Editorial

Inducible nitric oxide synthase in the cardiovascular system

Many acute and chronic diseases of the cardiovascular system are characterised by an inflammatory response. Atherogenesis is considered a chronic inflammatory disease of the vessel wall,¹ acute or chronic inflammation of the myocardium underlies certain forms of cardiomyopathy,² and severe acute inflammation secondary to infection (septic shock) leads to profound changes in the behaviour of blood vessels and the heart.³ An understanding of the processes underlying vascular inflammation should lead to novel approaches to treatment, and one pathway, the L-arginine:nitric oxide (NO) pathway, is currently arousing considerable interest as a fundamental mechanism of inflammation and a potential target for novel therapies.

Nitric oxide synthases

Three NO synthases have been identified—an endothelial type (eNOS), a neuronal type (nNOS), and a macrophage type that is known as inducible NOS (iNOS). Endothelial and neuronal NO synthases are normal constituents of healthy cells and synthesise NO to facilitate cell-cell communication. Both enzymes seem to have important roles in the regulation of the cardiovascular system and, paradoxically, both seem also to be “inducible” in response to certain stimuli including oestrogens.⁴ These two isoforms of NO synthase have been reviewed extensively elsewhere and are not considered further in this article.^{5,6} The third isoform, the classic inducible NOS (iNOS) is not a normal constituent of quiescent healthy cells but is expressed in a wide variety of cell types after they are exposed to bacterial endotoxin or combinations of inflammatory cytokines.^{7,8} Initially described in mouse macrophages, expression of this enzyme leads to prolonged synthesis of large amounts of NO. The NO produced by macrophages is an important component of what was known as “non-specific immunity” and is toxic to various pathogens including many protozoa⁹ and fungi,¹⁰ certain bacteria,¹¹ and viruses.¹² Host cells are not exempt from the toxic effect of NO and, while NO may be useful to inhibit growth and replication of abnormal cells including malignant cells, damage to healthy cells also occurs: endothelial cell damage and increased vascular permeability may result from overproduction of NO.^{7,13} Within the cardiovascular system, expression of iNOS has been implicated as a mechanism of tissue dysfunction and damage in several disease states.^{6,14–16} How strong is the evidence and are there opportunities for new therapies based on manipulation of iNOS activity?

Animal models

After exposure to bacterial endotoxin or certain cytokines (notably interleukin-1 and tumour necrosis factor α), expression of iNOS occurs throughout the cardiovascular system, in vascular endothelial cells,¹⁷ smooth muscle cells,¹⁸ endocardium,¹⁹ and macrophages located within the vessel wall.²⁰ Overproduction of NO in the blood vessel wall is associated with vasodilatation, resistance to con-

strictor stimuli, and endothelial cell damage or dysfunction.^{7,14} In the heart NO inhibits contraction in isolated cardiac myocytes,^{19,21} strips of papillary muscle, or intact perfused hearts.²²

Despite the clear biochemical and molecular biological evidence for the presence of iNOS protein, determining its contribution to pathophysiological changes has been less straightforward. Most of the inhibitors of NO synthesis that are currently available do not distinguish significantly between the three isoforms of NOS (eNOS, nNOS, and iNOS) and the overall effect produced by these compounds is a composite one, not due solely to inhibition of iNOS. However, recent experiments with inhibitors showing a degree of selectivity for iNOS have demonstrated the importance of this enzyme type in mediating the hypotensive and toxic effects of endotoxin.²³ Furthermore, “gene-knockout” experiments, in which the iNOS gene was disrupted in stem cells to produce adult mice lacking iNOS, have found that the mice without iNOS are more sensitive to certain infections with live organisms but resistant to the hypotensive and lethal effects of endotoxin.^{24,25} These studies confirm the role of induction of iNOS as a host defence mechanism and also clearly indicate its potential to damage the host, at least in endotoxaemia. Using similar techniques it should now be possible to identify more precisely the role of iNOS in models of chronic cardiovascular inflammation including atherogenesis and cardiomyopathy.

How does NO cause damage?

The mechanism by which expression of iNOS leads to cytotoxicity is uncertain. Many of the physiological effects of NO are mediated by interaction with the haem moiety of the enzyme guanylate cyclase and it is possible that toxic effects are due to interaction with haem or iron-sulphur centres of other enzymes.²⁶ Nitric oxide inhibits mitochondrial respiration,²⁷ damages DNA,²⁸ and inhibits replication of cells including vascular smooth muscle cells.¹⁸ At higher concentrations NO reacts rapidly with oxygen and in the presence of O_2^- (superoxide anion, another product of the inflammatory response) NO forms ONOO⁻ (peroxynitrite). Depending on the local environment and thiol concentration, peroxynitrite might either revert to NO, cause damage by leading to nitration, or lead to generation of other toxic radicals including OH[•] (hydroxyl radical).²⁹ It remains to be determined why normal physiological production of NO is protective in the cardiovascular system and may prevent atheroma formation^{30,31} whereas excess NO produced after expression of iNOS and under conditions of inflammation, is potentially harmful.

Inducible NOS in humans

The gene encoding iNOS in humans is located on chromosome 17³²; message for iNOS has been found in human hepatocytes³³ and vascular smooth muscle cells³⁴ exposed to cytokines or endotoxin, and immunohistochemistry has

identified the iNOS protein in macrophages³⁵ harvested from patients with inflammatory conditions. Expression of active iNOS has been demonstrated in cytokine-activated human vascular smooth muscle cells and endothelial cells in culture^{18,36} and addition of endotoxin to isolated blood vessels leads to vascular relaxation and functional changes consistent with expression of iNOS and overproduction of NO. Furthermore, there are biochemical and functional data to support a role for iNOS in the vasodilatation and hypotension of septic shock in patients.³⁷ Recently it has been suggested that iNOS is expressed in aneurysmal atherosclerotic human aorta¹⁷ and in the megakaryocytes of patients with atherosclerosis.¹⁶

There is therefore, evidence that iNOS can be expressed in the vessel wall of humans, produces biological effects, and is present under conditions of acute and chronic inflammation. iNOS activity has also been found in ventricular biopsies of patients with dilated cardiomyopathy² but experimental induction of iNOS in human myocytes or endocardial cells in response to endotoxin or cytokines has not yet been demonstrated. Studies with NO-donor drugs suggest that overproduction of NO in the human heart might decrease contractility and impair diastolic relaxation.³⁸

The precise role of iNOS in human cells has been a topic of considerable debate, but it has now been demonstrated that expression of iNOS in human macrophages leads to production of NO in amounts sufficient to kill pathogens,³⁹ suggesting that as in animals, iNOS is potentially an important host defence mechanism. Together, the data indicate that iNOS is expressed in the human cardiovascular system under conditions of inflammation, that the NO produced might be a mechanism of host defence, and that the effects of the expression of iNOS in the cardiovascular system include changes in cardiac and vascular function and tissue (including endothelial) damage. At least some of these effects are unwanted and potentially damaging to the host; a classic concept of immunopathology.

Therapeutic possibilities

Physiological production of NO from constitutive NOS in endothelium and nerves is important to maintain cardiovascular homeostasis⁶ and expression of iNOS seems to be important for host defence against intracellular pathogens. Currently there is therapeutic interest in boosting physiological levels of NO. Are drugs that inhibit NOS likely to do more harm than good? Available NOS inhibitors that have been used in humans do not distinguish between eNOS, nNOS, and iNOS. One of these, N^G-monomethyl-L-arginine (L-NMMA) has been given to patients with septic shock and it is clear that blocking NOS in sepsis restores blood pressure and arterial tone but cardiac output falls, probably in response to the increased arterial resistance.³⁷ However, in vivo and in vitro animal studies suggest that expression of iNOS in the heart itself contributes to the myocardial depression associated with sepsis and it would be important to establish the direct effect of inhibition of NO in the human heart in health and disease. Large trials of the clinical efficacy of L-NMMA in septic shock are underway and should determine whether non-specific inhibition of NOS will be of therapeutic benefit in this acute condition of vast overproduction of NO.

For other more chronic conditions, selective inhibitors of iNOS offer more promise. Certainly as this type of drug becomes more widely available for investigative use in animals²³ and eventually also in humans, it should be possible to determine the importance of iNOS in the pathogenesis

of damaging inflammatory reactions in the cardiovascular system including dilated cardiomyopathy and atherogenesis. However, even if chronic expression of iNOS has a major role in these conditions (and currently this seems plausible) it is unlikely that inhibitors of the enzyme will be free from problems: increased susceptibility to certain types of infection is a possible unwanted effect. The challenge will be to identify when the excess NO is providing protection and when it is causing damage to the host. The answer may lie in never aiming for more than incomplete inhibition of iNOS.

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