Apoptosis and atherosclerosis

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Apoptosis and Atherosclerosis: The Role of Nitric Oxide

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Abstract: Atherosclerosis, and its associated complications, are a major cause of morbidity and mortality, and it is now recognised as a chronic inflammatory disorder. Progression of inflammation depends on the balance between recruitment of inflammatory cells and their subsequent removal from a site of inflammation. Apoptosis, or programmed cell death, is a fundamental process governing cell survival and is a major determinant of the resolution of the inflammatory response. Apoptotic cells are instantly recognised for non-inflammatory clearance by phagocytes (e.g. macrophages) and removed from the vicinity of inflammation without the release of their pro-inflammatory cell contents. Nitric oxide (NO) plays an important role in many biological processes and has several anti-atherogenic properties including vasodilatation, inhibition of platelet activation and aggregation, and the regulation of apoptosis in a variety of cell types involved in atherogenesis. A critical early event during atherogenesis is injury to the endothelium. The ensuing damage results in endothelial dysfunction, including a reduction in the capacity of the endothelium to generate NO. Decreased NO bioavailability is likely to influence many cellular processes occurring within atherosclerotic lesions, including apoptosis. Modulation of apoptosis is a novel target for therapeutic intervention in the treatment of chronic inflammatory disorders, such as atherosclerosis. This modulation may help limit or resolve inflammation without the concomitant recruitment of subsequent inflammatory cells, thereby reducing the potential for further tissue damage. NO is a possible candidate for manipulation of atherosclerotic processes due to both its powerful anti-atherogenic characteristics and ability to affect apoptosis. This review highlights the role of apoptosis in atherosclerosis and discusses the therapeutic potential of NO to limit and/or resolve vascular inflammation.

Keywords: Atherosclerosis, nitric oxide, inflammation

INTRODUCTION

Atherosclerosis and its associated secondary complications, such as myocardial infarction and stroke, remain a major cause of morbidity and mortality in industrialised nations. Atherosclerosis is a multifactorial disease process with a complicated aetiology. However, it is now widely recognised that there is a chronic inflammatory component to the disease process, which is characterised by the formation of lipid-rich plaques in the wall of major conduit vessels such as the coronary arteries and aorta [1-4]. These lesions are usually eccentric and made up of a necrotic core of lipid-laden inflammatory cells encapsulated by a fibrous, collagen-rich cap consisting of vascular smooth muscle cells (VSMC) and extracellular matrix [5]. In this state, the plaque is considered 'stable'; its physical presence results in partial occlusion of the vessel, but because the vessel is dynamic rather than static, it can often compensate for this occlusion and accommodate for the presence of the plaque without a decrease in lumen diameter [6]. However, if the cap is subject to mechanical breakdown or erosion, the plaque can become 'unstable' and rupture. When the plaque cap is compromised, the highly thrombogenic contents of the core are released into the circulation. Platelets are rapidly recruited and activated resulting in thrombus formation. Plaque rupture can occur several times and remain sub-clinical with the VSMC cap reforming, or healing, over the top of the thrombus which becomes incorporated into the lesion, resulting in a layering effect within the plaque [7]. This process can further occlude the vessel *in situ*, or the thrombus can detach from the plaque surface, and the resulting embolus occlude smaller vessels downstream, leading to the more serious acute cardiovascular syndromes, such as myocardial infarction and stroke [8]. The determinants of plaque vulnerability to destabilisation and rupture have yet to be fully identified, but a growing body of evidence is emerging that points toward a critical role for both the thickness of the VSMC layer overlaying the core [9] and to inflammatory processes occurring within the plaque [10-12].

APOPTOSIS

Inflammatory cell apoptosis, or programmed cell death, is a highly regulated process whereby cellular death and subsequent phagocytosis occur without disruption of the cell membrane and ensuing release of the histotoxic and pro-inflammatory mediators from the cytoplasm [13-15]. Apoptosis therefore represents a non-inflammatory mechanism for the removal of cells from a site of tissue damage, and hence, is critical to the successful resolution of the inflammatory response. Pharmacological manipulation of apoptosis in a variety of cell types, particularly inflammatory cells, may represent a novel therapeutic strategy for the treatment of chronic inflammatory disorders, including atherosclerosis [16, 17].

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Apoptosis can be modulated by the endogenous messenger nitric oxide (NO), which can be both pro- and anti-apoptotic depending on local concentration, the specific cell type in question and the NO-related species generated *in vivo* [18, 19]. The aim of this review is to examine the role of apoptosis in atherosclerosis and to discuss the potential of this process as a target for therapeutic intervention by NO donor drugs, whereby disease progression may be stabilised in an effort to prevent the acute cardiovascular syndromes.

ATHEROSCLEROSIS

Despite arduous research in both humans and animal models of the process, the underlying causes of atherogenesis remain largely unknown. It is widely accepted that a critical early event of atheroma development is endothelial cell injury and damage. This can occur by several mechanisms: chemical injury can occur through oxidative stress, including oxidation of circulating low density lipids (LDL) resulting in the formation of damaging oxidised LDL (ox-LDL); and physical damage to the endothelium can result from shear stress within the vessel, with plaques tending to form at sites usually subjected to elevated shear stress, such as bifurcation points in the arterial tree or where low shear stress results in reduced stimulation of NO synthase and NO generation [20]. The consequences of an insult to the endothelium are numrous: firstly, the damaged endothelium becomes dysfunctional and the net production of NO by eNOS decreases, promoting vasoconstriction. This is reflected in patients with risk factors for atherosclerosis, such as hypercholesterolaemia. In hypercholesterolaemic individuals, vasodilatation in response to the endothelium-dependent vasodilator, acetylcholine (which acts by triggering NO release from the endothelium), is impaired in forearm resistance vessels compared to normocholesterolaemic controls. However, vasodilatation in response to the endothelium-independent NO donor, sodium nitroprusside (SNP), is unaffected. This suggests that endothelial injury resulting from the hypercholesterolaemic state decreases the normal NO-producing capacity of the endothelium [21]. Although resistance vessels are not usually susceptible to plaque formation, they are widely accepted to reflect changes occurring globally throughout the arterial tree.

In addition, in response to the insult to the endothelium, an inflammatory response is triggered. Depending on the nature and duration of the insult, this response can become excessive and, over a period of years, constitutes the disease process itself [2, 3]. The inflammatory process begins with the expression of chemotactic and adhesion molecules for monocytes and lymphocytes, including vascular cell adhesion molecule 1 (VCAM-1), on the surface of dysfunctional endothelial cells. Circulating monocytes adhere to the site of endothelial damage and translocate to the sub-endothelial space where they accumulate [22]. Colony stimulating factors secreted from areas of endothelial damage induce monocytes to differentiate into macrophages, which then begin to express scavenger receptors, facilitating the internalisation of oxidised low density lipoprotein (ox-LDL) [23]. Cultured macrophages do not accumulate native LDL on account of downregulation of LDL receptors in the cholesterol-replete state. However, once LDL has been modified by oxidation, accumulation of the resultant ox-LDL by macrophages continues unchecked because scavenger receptors are not downregulated by cholesterol accumulation. The mechanism of lipid peroxidation is still not fully understood, but free radicals such as superoxide (O_2^-) and hydroxyl radicals ($^{\bullet}OH$), undoubtedly have a role to play [24, 25]. Peroxynitrite ($ONOO^-$), formed by the rapid reaction of NO with O_2^-, can also initiate lipid peroxidation, both *in vitro*, and in membrane lipids and lipoproteins [26, 27]. In the lipid-laden condition, macrophages are known as foam cells and it is an accumulation of these cells in the vessel intima which forms the earliest recognisable lesion of atherosclerosis – the fatty streak (Fig. **1A**) [20, 28]. Fatty streaks have been observed in the vessels of young adults and children (including neonates), suggesting that atherosclerosis may be initiated early in life but remains sub-clinical unless vessel occlusion and/or plaque rupture lead to diagnosis [29].

Once the fatty streak is established, the plaque grows in size as the ox-LDL accumulated in macrophage-derived foam cells causes both further endothelial damage and is chemoattractant for circulating monocytes, which freely migrate through the endothelium, accumulate in the sub-endothelial space, and differentiate into macrophages which then go on to become foam cells [30]. This establishes a perpetual cycle of endothelial damage, leading to monocyte recruitment and accumulation of ox-LDL, which encourages further endothelial damage (Fig. **1**). Activated macrophages in fatty streaks secrete numerous cytokines and growth factors including platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF), interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β) [20]. All of these factors and cytokines induce vascular smooth muscle cell (VSMC) hypertrophy and/or hyperplasia. This represents a change in phenotype for VSMCs, from the usual adult vascular contractile phenotype to the synthetic phenotype usually seen only in developing vessels. However, characterisation of VSMCs into only two distinct phenotypes is a vast over-simplification. A wide spectrum of diverse intermediary phenotypes exist under different physiological and pathophysiological conditions, without a clear distinction between phenotypes. This is especially true when VSMCs are undergoing phenotypic transition and there may, in fact, be several sub-populations of VSMCs present within the plaque at any given time [31]. In a synthetic phenotype, VSMCs have the ability to proliferate and synthesise large amounts of collagen. Proliferation begins in the media of the vessel, but after a time the cells begin to migrate, invade the intima and form a layer over the top of the fatty streak. This layer of VSMCs secretes large amounts of extracellular matrix and connective tissue consisting of glycosaminoglycans, dermatan sulfate, and collagen. These molecules form a mesh over the fatty streak, which becomes calcified and forms the fibrous cap of the plaque, encapsulating the highly thrombogenic lipid core and maintaining a barrier between the plaque contents and the circulation (Fig. **1A**) [4, 5].

APOPTOSIS IN ATHEROSCLEROSIS

Inflammatory Cells

Recruitment of inflammatory cells, particularly monocytes and macrophages, is the major driving force behind

plaque growth and development. Apoptosis of inflammatory cells within an atherosclerotic lesion may result in their subsequent phagocytosis and removal from the plaque core without the concomitant recruitment of additional inflammatory cells (Fig. **1B**). The identity of the cells responsible for clearing the apoptotic macrophages from the plaque remains to be confirmed. It is currently unclear whether activated macrophages can themselves phagocytose populations of apoptotic inflammatory cells, or if this is done by a separate subset of specialised phagocytes.

Apoptotic macrophages have been located by TUNEL staining in plaques from animal models of atherosclerosis and in plaques excised from human vessels [32-34]. The apoptotic macrophages tend to be clustered in areas of the plaque most vulnerable to rupture, most notably the base, or shoulder, regions where the VSMC prevalence is also decreased [35]. It is currently unclear why these areas of the plaque are particularly susceptible to rupture, however, reduced VSMC localisation in these areas of vulnerable plaques may indicate that apoptosis of a variety of cell types, including VSMCs, may play a role in plaque susceptibility to rupture (Fig. **1C**) [36]. Recently, it has been suggested that the macrophage enzyme, myeloperoxidase (MPO), could also have an important role in determining plaque vulnerability. MPO, a member of the heme peroxidase superfamily, generates reactive oxidants including hypochlorous acid (HOCl) as part of its normal function in innate host defences [37, 38]. Sugiyama *et al.* have described a strong co-localisation between macrophage MPO expression and HOCl-modified proteins at sites of lesion rupture in patients who suffer acute cardiac events [39]. MPO-generated HOCl at relatively high physiological concentrations, but still within the range expected to be produced at areas of vascular inflammation (30 – 50 μM), has been shown to promote endothelial cell death by stimulating apoptotic pathways including rapid caspase-3 activation and DNA fragmentation [40]. This observation suggests that, prior to undergoing apoptosis themselves, activated macrophages may induce apoptosis of neighbouring endothelial cells through MPO expression, resulting ultimately in plaque rupture.

Vascular Smooth Muscle Cells

As previously mentioned, loss of VSMCs from the protective plaque cap is a major determinant of plaque rupture. Because healthy endothelial cells secrete factors that promote VSMC survival, a consequence of activated macro-

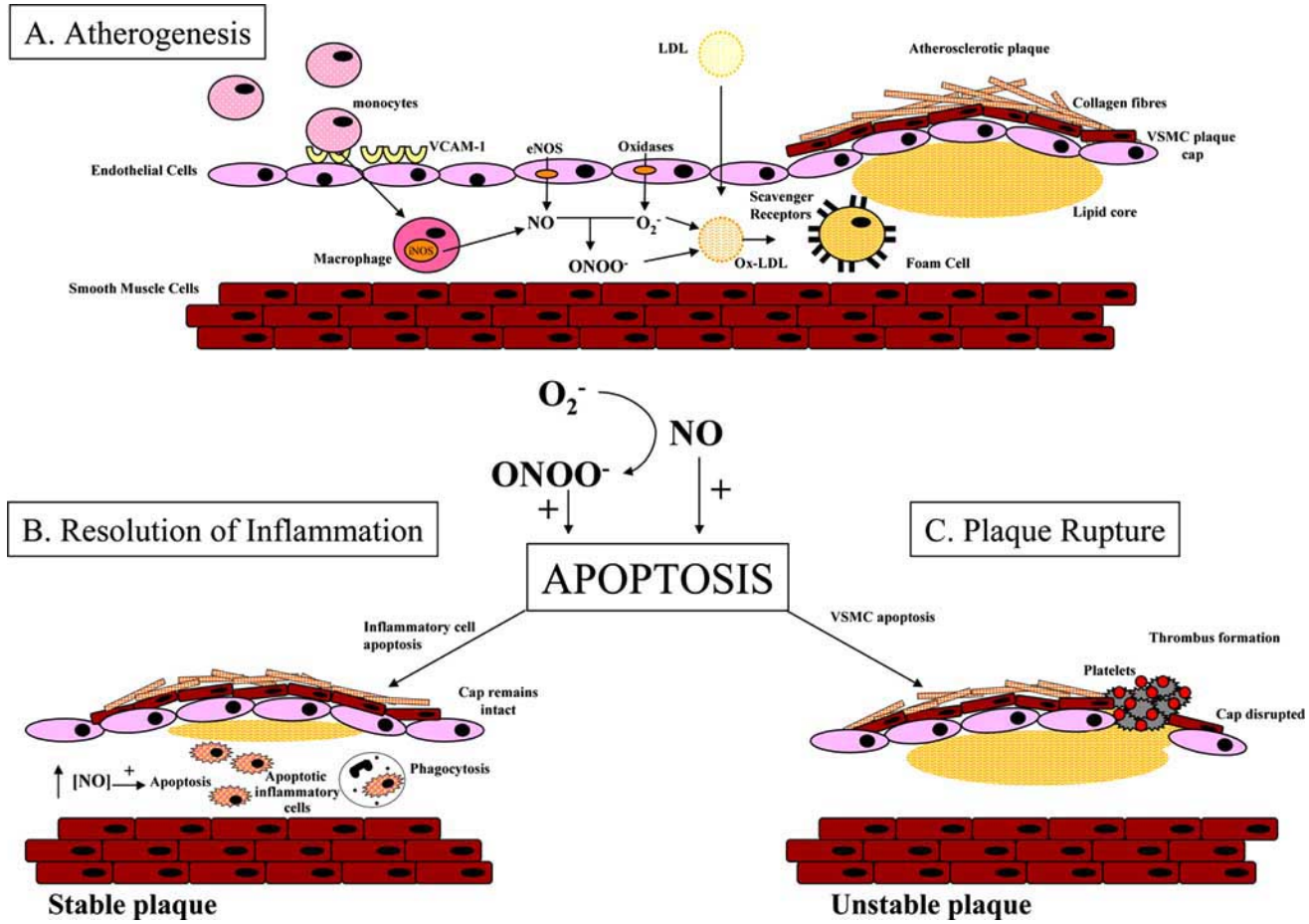

Fig. (1). During atherogenesis circulating monocyes translocate to the subendothelial space where they accumulate modified lipids and form an atherosclerotic plaque, overlain with a fibrous cap of VSMCs (A). Apoptosis can be induced in a variety of cell types by NO. Apoptotic inflammatory cells are cleared by phagocytes, aiding resolution of the inflammatory response, and ultimately facilitating plaque regression (B). However, apoptosis of VSMC may be detrimental, resulting in degradation of the plaque cap and leading to plaque rupture, particularly in the shoulder regions, and ultimately to thrombus formation (C).

phage-induced endothelial cell death is an increase in VSMC death [41]. Direct induction of VSMC apoptosis in the apolipoprotein E null murine model of atherosclerosis induces both rupture and thrombosis of the plaque [42]. In addition to removing the protective presence of the endothelium, macrophages can also influence VSMC apoptosis directly. Activated human monocytes/macrophages have been found to be responsible for the death of human VSMCs *in vitro* [43, 44]. Seshiah *et al*. have hypothesised that macrophage colony-stimulating factor (M-CSF), a haematopoietic growth factor supporting survival and differentiation of monocytes, is secreted from VSMCs resulting in macrophage activation, ultimately triggering subsequent VSMC apoptosis [44]. The exact mechanism of this process remains to be fully understood but is thought that macrophages prime VSMCs to respond to apoptotic stimuli, for example by triggering the expression of death receptor ligands such as TNF-α on the cell surface [45].

NITRIC OXIDE

NO was first identified as the biological messenger responsible for endothelium dependent vasodilatation [46, 47]. Since then, it has become increasingly clear that NO has many diverse actions beyond that of control of vascular tone. Synthesised by three isoforms of the enzyme NO synthase (NOS), NO is now known to inhibit platelet and inflammatory cell adhesion and activation, to act as a transmitter at non-adrenergic non-cholinergic (NANC) neurones, and to exhibit both pro- and anti-apoptotic effects depending on the NO-related species, concentration and cell type in question [48].

The classical pathway by which NO exerts its effects is *via* activation of the enzyme soluble guanylate cyclase (sGC) and resultant conversion of guanosine 5'-triphosphate (GTP) to 3', 5'-cyclic guanosine monophosphate (cGMP) [48, 49]. However, recent studies have established that NO can act *via*, as yet unidentified, cGMP-independent pathways in various systems, particularly during the inhibition of platelet aggregation [50-53] and regulation of inflammatory cell apoptosis [51-54].

There is currently much debate as to whether all of the effects of NO are mediated by the NO radical *per se*, or by other NO-related species formed *in situ.* Due to its free radical status, NO is highly reactive, and combines readily with reactive oxygen species (ROS) to form a variety of NO-related species. For example, superoxide anions (O_2^-), often generated at sites of inflammation by the inflammatory cell enzyme NADPH oxidase [55], react rapidly with NO to produce toxic peroxynitrite ($ONOO^-$). This reaction is usually prevented *in vivo* by the battery of antioxidants that inactivate ROS. For instance, the enzyme superoxide dismutase (SOD) removes O_2^- by catalysing its conversion to hydrogen peroxide (H_2O_2), which is subsequently further inactivated by catalase [56]. However, if the production of ROS is such that the antioxidant capacity is overcome, then any free NO present will react rapidly with the O^-_2 to form $ONOO^-$. NO can also form S-nitrosothiols *via* the transfer of NO^+ from higher oxides of nitrogen (e.g. N_2O_3) to reduced thiol groups on proteins such as albumin, resulting in the formation of S-nitrosoalbumin [57, 58]. It has recently been hypothesised that the formation of S-nitrosothiols may act as a slow-release NO store *in vivo* [50]. S-nitrosation of a range of cellular proteins is now considered to be one of a number of post-translational modifications that can alter protein function, and these modifactions are likely to be responsible for many of the cGMP-independent effects of NO, including inhibition of caspase-3 activation [59, 60].

NO AS A MEDIATOR OF APOPTOSIS

The pro- and anti-apoptotic actions of NO have been well documented in many cell systems. Current evidence suggests that lower concentrations of NO produced constitutively by endothelial and neuronal NOS (eNOS and nNOS) are cytoprotective *via* primarily cGMP-dependent mechanisms, whilst higher, supraphysiological concentrations generated in some pathologies by inducible NOS (iNOS) mediate apoptosis *via* mechanisms independent of cGMP signalling [61]. For example, high concentrations of either exogenous or endogenous iNOS-derived NO have been shown to induce apoptosis in murine and rabbit macrophage cell lines [62, 63]. Apoptosis in rabbit macrophages was unaffected by inhibition of cGMP-dependent kinases and not mimicked by cGMP analogs, suggesting that the pro-apoptotic actions of NO are cGMP-independent [64]. Conversely, pre-treatment with relatively low concentrations of exogenous NO, delivered by the synthetic NO donor sodium nitroprusside (SNP) protects RAW 264.7 cells (a mouse macrophage cell line) against cell death upon subsequent exposure to higher concentrations of NO which would normally be cytotoxic [65]. Furthermore, this protection was mimicked by cGMP analogs but negated in the presence of sGC inhibitors, suggesting that the cellular protection was conferred by cGMP. Similarly, high levels of NO produced by activated macrophages as a consequence of iNOS upregulation may also induce VSMC apoptosis through DNA damage and subsequent p53 activation [66].

This dual role, and apparent paradox of NO, may be explained, at least in part, by the free radical nature of NO and the ease with which it will form various NO-related species *in vivo*. As already mentioned, NO will react rapidly with O_2^- to form $ONOO^-$. The production of $ONOO^-$ may be of particular importance at sites of vascular inflammation such as atherosclerotic lesions, where the concentration of reactive oxygen is likely to be elevated [67]. The precise role of $ONOO^-$ in inflammatory cell apoptosis remains to be elucidated. There is some evidence to suggest that at high concentrations (100 μM – 300 μM), $ONOO^-$ induces apoptosis in RAW 264.7 cells [68], whilst at lower concentrations (30 μM – 50 μM), it may have a protective effect against lipopolysaccharide (LPS) and interferon (IFN)-γ-induced apoptosis in these cells [69]. Brockhaus *et al.* [70] have demonstrated that overexpression of copper/zinc SOD (CuZnSOD) can protect RAW 264.7 cells against apoptosis initiated by NO, either exogenous or iNOS-derived. This implies a role for $ONOO^-$ as the mediator of NO-initiated apoptosis. However, whilst a specific scavenger of $ONOO^-$, uric acid, effectively abolishes apoptosis induced by the $ONOO^-$ generator SIN-1, it left apoptosis induced by the NO donors S-nitrosoglutathione (GSNO) and spermine diazeniumdiolate SPER/NO unaltered, suggesting that NO and NO-related species

may be able to activate several pathways when initiating apoptosis [70].

Whilst endogenous macrophage iNOS-derived NO has been shown to induce apoptosis in animal models, this is not necessarily the case in human macrophages, which might not produce any NO at all. [71-73]. However, despite this reduced capability to produce endogenous NO, human macrophages still undergo apoptosis in response to exogenous NO and NO-related species. For example, the NO donors GSNO and SPER/NO induce apoptosis in primary human monocyte-derived macrophages [74].

NO AND APOPTOSIS IN THE RESOLUTION OF INFLAMMATION

Apoptosis is now thought to be key to the resolution of the inflammatory response. Pharmacological manipulation of the rate of apoptosis during chronic inflammatory disorders such as atherosclerosis, may aid the resolution of inflammation and delay disease progression. NO is a promising candidate for such manipulation, because its ability to induce apoptosis and aid inflammatory resolution has already been demonstrated in several animal models. In a mouse model of kidney inflammation, activated macrophages have been shown to induce apoptosis in neighbouring mesangial cells prior to ingestion by phagocytes [75]. The ability of the activated macrophages to induce apoptosis is greatly reduced in the presence of the NOS inhibitor, N^{G}-monomethyl-L-arginine (L-NMMA), indicating that macrophage-directed apoptosis of mesangial cell apoptosis occurs *via* NO-dependent mechanism [76]. Similarly, several studies have demonstrated that activated macrophages infiltrating murine tumours induce apoptosis *via* a NO-dependent pathway in both activated anti-tumour T cells and in the tumour cells themselves [77, 78]. Thus, it appears that macrophages have the capacity to induce apoptosis of nearby cells by the liberation of NO, or a related species, to enhance the clearance of apoptotic cells.

Inducing apoptosis in inflammatory cells within an atherosclerotic lesion is an attractive prospect as it may represent a mechanism to stabilise the plaque or to resolve the vascular inflammation and lead to regression of the plaque, thereby halting disease progression. Reducing the number of activated macrophages present in the plaque would have multiple consequences. Firstly, the physical presence of the plaque in the vessel wall would be decreased, reducing stenosis. Secondly, because activated macrophages induce apoptosis in neighbouring endothelial cells and VSMCs, reducing the number of activated macrophages may help to preserve endothelial function and maintain the integrity of the plaque cap.

NO as a mechanism to regress atherosclerosis is an appealing possibility because in addition to the pro-apoptotic properties described above, NO also has a number of other powerful anti-atherogenic characteristics including inhibition of platelet and inflammatory cell activation [48, 79]. Animal studies are emerging to support the hypothesis that manipulation of apoptosis could be used to reverse atherosclerosis. For example, L-arginine (the substrate for NOS) or the NO donor SNP, administered to hypercholesterolaemic rabbits increases the number of apoptotic macrophages in intimal lesions by three fold [64]. This increase in apoptosis was accompanied by a significant reduction in lesion surface area, suggesting that manipulation of the NO synthase pathway, or delivery of exogenous NO, may be a way to boost NO availabilty in order to stabilise, or even regress, the plaque *via* an apoptotic mechanism. However, the treatments used in this study are by no means selective for macrophages, and as already discussed, NO will also induce apoptosis in endothelial cells and VSMCs. This could have several serious detrimental consequences for the plaque; firstly, additional loss of endothelial function would occur, leading to further exacerbation of the disease process. Secondly, because VSMCs are essential for maintaining the integrity of the plaque cap, loss of this population of cells in vulnerable areas of the lesion could de-stabilise the plaque resulting in plaque rupture which could result in myocardial infarction or stroke. The potential benefit of regressing the plaque in this way must also be offset against the cost of reducing the size of the macrophage population available for scavenging existing apoptotic macrophages, endothelial cells and VSMC. If left *in situ*, these apoptotic cells will undergo secondary necrosis, thereby increasing the thrombogenicity of the plaque as a whole. In the study by Wang *et al.* [64] discussed above, apoptosis is quantified by the number of apoptotic nuclei per area of plaque, rather than as a percentage of the total macrophage population. Therefore, it is not possible to draw any conclusions as to whether the phagocytosis capacity of the plaque has been effected in this case.

Although human macrophages appear unable to generate the supraphysiological concentrations that murine cells produce, human macrophages will respond to exogenous NO delivered by synthetic NO donor compounds, suggesting that NO could potentially be used to manipulate rates of apoptosis in human atherosclerosis. However, it is essential to target any therapeutic intervention to specific cell types within the plaque, and in particular, to appropriate cell types within plaques vulnerable to rupture. Indiscriminate pro-apoptotic events may have serious adverse consequences for the plaque dynamic.

At present, existing NO donor drugs are not selective for particular cell types. Common clinically used organic nitrates, such as nitroglycerin (NTG), tend to have an unfavourable selectivity profile when considered in the context of atherosclerosis, they are more selective for veins (which are not subject to plaque formation) than arteries, and more selective for arteries than platelets (which play a major role in thrombus formation following plaque ruture). In order to use NO donor drugs as effectors of apoptosis in atherosclerosis, compounds which are able to selectively act on, for example, macrophages but not endothelial cells or VSMC, will have to be developed in the future. This however, may be difficult given that macrophages are relatively resistant to oxidative stress induced apoptosis.

In addition to selectivity, a major consideration when contemplating the use of NO donor drugs for the treatment of atherosclerosis is that of delivery. Global, non-selective release of NO throughout the circulation may have various anti-atherosclerotic actions, but dosing will be limited by concurrent vasodilatation resulting in systemic hypotension. The challenge therefore, is to generate high local concentra-

tions in the vicinity of a plaque. One possible means of achieving this is to employ NO donor drugs which are selective for areas of endothelial damage. S-nitrosothiols are generally accepted to be platelet-selective NO donor drugs [80] and have been shown *in vitro* to have vasodilator actions which are selective for areas of experimentally denuded endothelium [81]. Furthermore, in a rabbit balloon angioplasty model of vascular injury, the S-nitrosothiol, S-nitroso-*N*-valerylpenicillamine (SNVP), in contrast to the traditional organic nitrate NTG, reduced the adhesion of radio-labelled platelets in areas of endothelial damage without significantly affecting systemic blood pressure [82]. In this study, the NO donors were delivered directly into the carotid artery *via* a cannula; obviously this is not practical in humans as a long - term therapy, but a possible alternative is to deliver NO *via* drug-eluting stents. There has been some success in humans with the use of sirolimus (rapamycin)-eluting stents in preventing neointimal proliferation (which can lead to restenosis) following angioplasty, although long-term effectiveness (>1 year) remains to be established [83]. A further potential method of delivering high local concentrations of NO directly to the interior of the plaque may be to exploit the lipid nature of the plaque core by developing novel lipophilic NO donor drugs designed to induce apoptosis in the lipid-laden macrophages within the core.

CONCLUSION

Atherosclerosis is now considered a chronic vascular inflammatory disorder involving complicated interactions between many cell types. Apoptosis plays a key role in determining both turnover of cells within the plaque, and plaque vulnerability to rupture. Because apoptotic cells are removed from the site of inflammation without triggering a subsequent pro-inflammatory response, inflammatory cell apoptosis is a promising target for therapeutic intervention in the treatment of this disease. The ability of NO to induce apoptosis, combined with the additional anti-atherogenic properties of NO, points to this molecule being a powerful tool in the treatment of atherosclerosis. The challenge will be to deliver NO in the appropriate chemical form and to balance any benefit in reducing inflammatory cell number within the plaque against the potential deleterious effects of destabilising the plaque cap and promoting plaque rupture.

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