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THE SIMULTANEOUS GENERATION OF SUPEROXIDE AND NITRIC OXIDE CAN INITIATE LIPID PEROXIDATION IN HUMAN LOW DENSITY LIPOPROTEIN

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Oxidation of low density lipoprotein (LDL) has been shown to occur in the artery wall of atherosclerotic lesions in both animal models and human arteries. The oxidant(s) responsible for initiating this process are under intensive investigation and 15-lipoxygenase has been suggested in this context. Another possibility is that nitric oxide and superoxide, generated by cells present in the artery wall, react together to form peroxynitrite which decomposes to form the highly reactive hydroxyl radical. In the present study we have modelled the simultaneous generation of superoxide and nitric oxide by using the sydnonimine, SIN-1 and have investigated its effects on LDL. SIN-1 liberates both superoxide and nitric oxide during autooxidation resulting in the formation of hydroxyl radicals. We have demonstrated that superoxide generated by SIN-1 is not available to take part in a dismutation reaction since it reacts preferentially with nitric oxide. It follows, therefore, that during the autooxidation of SIN-1 little or no superoxide, or perhydroxyl radical will be available to initiate lipid peroxidation. We have shown that SIN-1 is capable of initiating the peroxidation of LDL and also converts the lipoprotein to a more negatively charged form. The SIN-1-dependent peroxidation of LDL is completely inhibited by superoxide dismutase which scavenges superoxide. Neither sodium nitroprusside or S-nitroso-n-acetyl penicillamine, which only produce nitric oxide, are able to modify LDL. These results are consistent with the hypothesis that a product of superoxide and nitric oxide could oxidize lipoproteins in the artery wall and so contribute to the pathogenesis of atherosclerosis *in vivo*.

KEY WORDS: Atherosclerosis, peroxynitrite, superoxide, nitric oxide, peroxidation.

ABBREVIATIONS: LDL (low density lipoprotein), SNAP (S-nitroso-n-acetylpenicillamine), SNP (sodium nitroprusside), PBS (phosphate buffered saline), TBARS (thiobarbituric acid reactive substances), TNBS (tri-nitrobenzene sulphonic acid), SOD (superoxide dismutase), R.E.M. (relative electrophoretic mobility) DTPA (diethylenetriaminepentaacetic acid).

INTRODUCTION

It is now becoming clear that the products of lipid peroxidation can be highly cytotoxic and could play a major role in mediating the pathophysiology of a number of diseases.[1] Perhaps the best documented example is the process of atherosclerosis in

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which oxidation of low density lipoprotein (LDL) occurs in atherosclerotic lesions and is associated with decomposition of the lipids in the lipoprotein to form aldehydes.[1-3] Lipid peroxidation is generally thought to be composed from two kinetically and mechanistically distinct reactions, namely initiation, in which a radical is formed from a lipid substrate, and propagation, in which peroxidation is sustained by lipid-derived peroxyl radicals. The reaction ceases after the substrates required to drive it are consumed. Inhibition of propagation can be achieved by chain breaking antioxidants which can react directly with peroxyl radicals. Since the products of LDL peroxidation appear to be similar for oxidation *in vivo* and *in vitro* it is likely that the mechanism for propagating this reaction is the same in both situations and involves the peroxyl radical.[2-4] The mechanism of initiation of lipid peroxidation in biological systems is unknown and is the subject of much continuing speculation. Decomposition of preformed peroxides, perhaps generated by the enzymatic activity of 15-lipoxygenase, by transition metals or by haem proteins within the artery wall is one route by which propagation could occur.[5,6] Alternatively, it is possible that a powerful oxidant such as the hydroxyl radical abstracts a hydrogen atom from the alkyl chain of a fatty acid to form an alkyl radical which will then react rapidly with oxygen to form the peroxyl radical.[7] The peroxyl radical is then capable of sustaining a lipid peroxidation reaction. This hypothesis requires a source of hydroxyl radicals *in vivo*.

Recently, a novel mechanism for the production of the hydroxyl radical has been proposed which involves the reaction of the superoxide radical with nitric oxide to form peroxynitrite.[8-10] In experiments where pure peroxynitrite has been used it was shown that this molecule can decompose spontaneously to form the hydroxyl radical and initiate the oxidative modification of both lipids and proteins.[9,10] Consistent with this hypothesis we have recently shown that the simultaneous generation of superoxide and nitric oxide by the sydnonimine, SIN-1, results in the formation of hydroxyl radicals by a mechanism which has no requirement for transition metals.[11] A system which simultaneously generates superoxide and nitric oxide offers several advantages over the use of chemically synthesised, pure peroxynitrite. For example, it is clear that, in principle, both nitric oxide and superoxide can take part in reactions which would not result in the formation of potent oxidants. Among these are the dismutation of superoxide to form hydrogen peroxide and the direct reaction of nitric oxide with oxygen to form nitrite. Central to the hypothesis that peroxynitrite-dependent oxidant production contributes to the pathophysiology of conditions such as ischaemia-reperfusion, is the premise that, under physiological conditions, there is a significant rate of reaction between nitric oxide and superoxide. The sydnonimines, a class of compounds used as vasodilators, are ideal in this respect since they produce nitric oxide and superoxide in solution at comparable rates.[12,13] Here we show that SIN-1 is capable of initiating lipid peroxidation in LDL while SNP and SNAP, which are compounds capable of generating only nitric oxide, cannot oxidise LDL.

MATERIALS AND METHODS

Human LDL was prepared by ultracentrifugation as described before[14] and dialysed against phosphate buffered saline containing 10 μM ethylene diamine tetraacetic acid (EDTA). The protein concentration was determined by using the BCA method in kit form as supplied by Pierce and was typically 1–2 mg protein/ml. Samples of LDL were

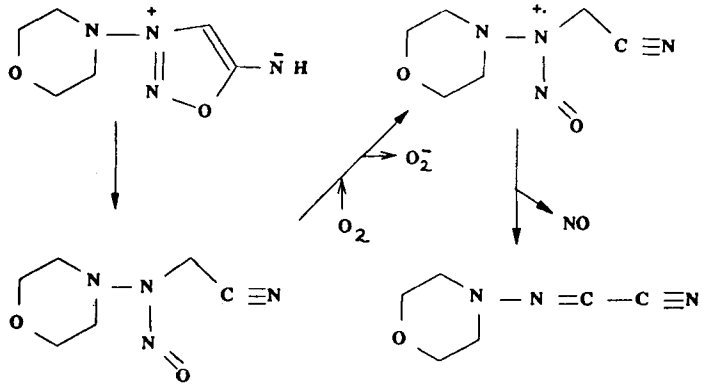

FIGURE 1 The autooxidation of SIN-1 showing the concomitant formation of superoxide and nitric oxide (adapted from Ref. 13).

diluted to the concentration reported for each experiment without further manipulation. Lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS) were measured as described in Refs. 15 and 16 respectively. Electrophoretic mobility of LDL samples was measured on agarose gels using the lipoprotein electrophoresis system supplied by the Beckman company. Stock solutions of SIN-1 were prepared in Ca^{2+}/Mg^{2+}-free phosphate buffered saline (PBS) just before use. Superoxide dismutase, purchased from the Sigma chemical company, was either used as supplied or substantially inactivated by boiling for 30 min. The remaining activity of the boiled enzyme was measured as described in Ref. 17. The SIN-1-dependent uptake of oxygen was measured in a Clark-type oxygen electrode supplied by the Rank Brothers, Cambridge. The number of primary amino groups in LDL before and after treatment with SIN-1 was determined after reaction with tri-nitrobenzene sulphonic acid (TNBS) using the amino acid valine as a standard as described in Ref. 18. Vitamin E levels were measured in LDL samples by HPLC after extraction with n-heptane exactly as described in Ref. 19.

RESULTS

The Effects of Superoxide Dismutase (SOD) and Catalase on Oxygen Consumption by SIN-1

It is well documented[11–13] that the autooxidation of SIN-1 results in the consumption of oxygen and this can be monitored using the oxygen electrode. After a short lag period, the rate of consumption of oxygen by SIN-1 (1 mM), at 37°C and pH 7.4, is constant at approximately 7–8 μM/min and only decreases when the oxygen concentration drops below approximately 100 μM. The proposed route for the decomposition of SIN-1 is shown in Figure 1. The lag period is thought to be due to the production of SIN-1A from SIN-1 (see Figure 1) which involves the base-catalysed ring opening of SIN-1.[14] SIN-1A is oxidised by oxygen to produce superoxide and a nitrogen-centred free radical which decomposes to nitric oxide and SIN1-C. The consumption of oxygen by SIN-1 could be due to both the oxidation of SIN-1A, as shown in Figure 1, and the reaction of nitric oxide with oxygen to produce nitrite.[12,13]

TABLE I
The effect of SOD and catalase on the SIN-1-dependent consumption of oxygen

Addition	O_2 uptake μM/min	O_2 uptake (as % of control)
SIN-1	7.10 ± 0.96	100
SIN-1 + SOD (50 U/ml)	4.53 ± 0.72	64
SIN-1 + Cat (500 U/ml)	6.95 ± 1.26	98

SIN-1 (1 mM) was incubated in PBS containing 0.1 mM DTPA, at 37°C, in the chamber of an oxygen electrode. The maximum rate of oxygen consumption was measured, after a short lag time of 2–4 min, under the conditions shown. Results are expressed as the mean ± standard deviation for three experiments.

The reaction of nitric oxide with superoxide has been clearly demonstrated in a number of systems, including the decomposition of SIN-1.[12,13] In order to assess the relative contributions of these reactions to the SIN-1-dependent consumption of oxygen, catalase and SOD were used as scavengers of hydrogen peroxide and superoxide respectively and their effect on the rate of oxygen consumption measured (Table 1). We found that whereas SOD inhibited the rate of oxygen uptake by approximately 36%, catalase had no effect. This suggests that the spontaneous dismutation of superoxide to form hydrogen peroxide is not occurring to any significant degree during the autooxidation of SIN-1. These results are consistent with the hypothesis that the reaction of superoxide with nitric oxide competes effectively with the uncatalysed dismutation of superoxide. This would explain the lack of effect of catalase on the rate of the SIN-1-dependent oxygen uptake and is consistent with the published rate constants for the reaction of superoxide and nitric oxide of $3.4 \times 10^7\,M^{-1}s^{-1}$, at 20°C, compared with the rate constant for the spontaneous dismutation of superoxide which is $5 \times 10^5\,M^{-1}s^{-1}$ at 37°C.[20] SOD increases the rate of this latter reaction to approximately $10^9\,M^{-1}s^{-1}$ and so only in the presence of this enzyme will superoxide dismutation effectively compete with the reaction of superoxide and nitric oxide.

The Effect of SIN-1 on LDL

SIN-1 was incubated with isolated human LDL (200 μg/ml) for a period of 16 h at 37°C in the presence of 100 μM DTPA, after which samples were cooled to 4°C, their lipid peroxide content measured and electrophoretic mobility determined relative to native LDL. These parameters are shown plotted as a function of the SIN-1 concentration in Figure 2A. It is clear that both the lipid peroxides and relative electrophoretic mobility (R.E.M.) increase as a function of the initial SIN-1 concentration up to 1 mM after which a slight decrease occurs. In order to determine the optimum time for incubation of SIN-1 with LDL to achieve the maximum degree of oxidation samples were incubated with 1 mM SIN-1 for 1–24 h and the extent of lipid peroxide formation and change in electrophoretic mobility determined. The results of this experiment are shown in Figure 2B. In the early stages of the reaction, oxidation of the lipid phase of the LDL precedes the change in electrophoretic mobility. On the basis of this data we chose conditions of 1 mM SIN-1 and incubation for a period of 16–24 h for subsequent experiments. These experiments were repeated for a number of preparations of LDL isolated from different donors and gave essentially the same result with R.E.M. ranging from 2.2–2.7 after 24 h oxidation (Table II). This small difference is most likely due to the well documented variation in the susceptibility of LDL isolated from different donors to oxidation.[21,22] The conversion of native LDL

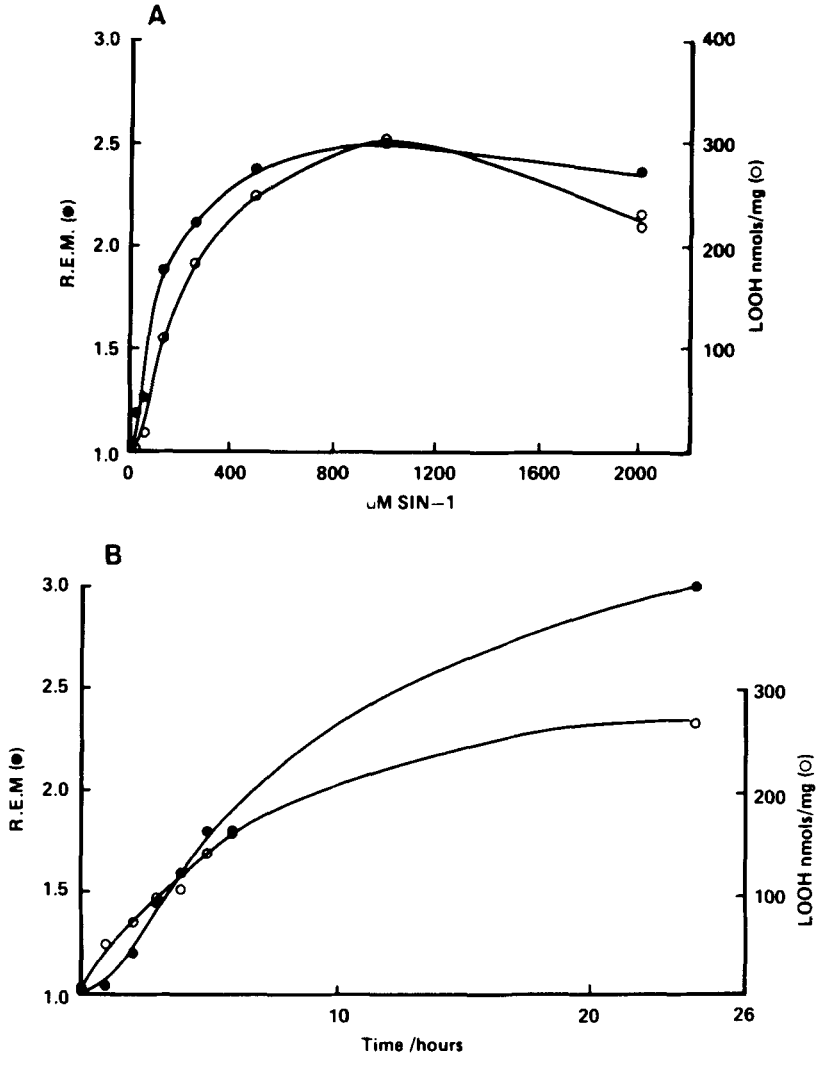

FIGURE 2 Effect of SIN-1 on the electrophoretic mobility of LDL and peroxide content. Panel A: LDL (200 μg/ml) was incubated with a range of SIN-1 concentrations after which the lipid peroxide (LOOH) content and electrophoretic mobility relative to native LDL (R.E.M.) was determined. Panel B: LDL was incubated with 1 mM SIN-1 for the time period shown after which samples were taken for measurement of R.E.M. or peroxide content. The results shown are the mean of duplicate measurements for a typical experiment.

to a more negatively charged form is generally ascribed to the modification of lysine residues on the apo-B protein and this can occur as a result of decomposition of the lipid hydroperoxides to form aldehydes.[18,23] It is thought that the aldehydes then modify positively charged amino acids on the LDL molecule and elicit the conformational change in the particle which renders it an avid ligand for the macrophage

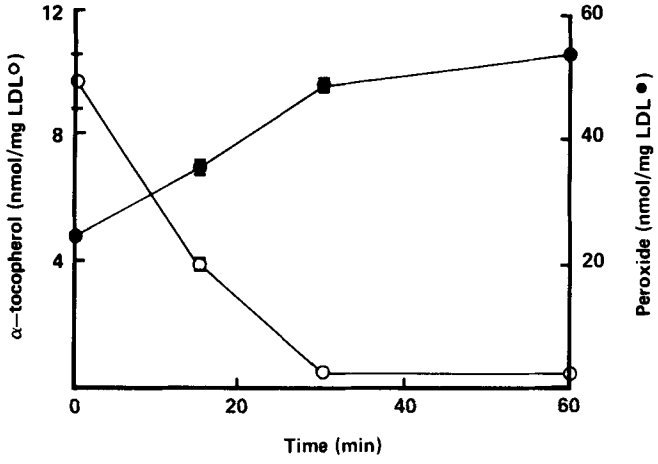

FIGURE 3 Effect of SIN-1 on levels of α-tocopherol and peroxide content. LDL (200 μg/ml) was incubated with 1 mM SIN-1 at 37°C for the times indicated after which the peroxide content (●) of the samples was determined and the tocopherol levels (○) measured. The data shown are the means ± S.D. of triplicate determinations of the same sample.

scavenger receptor.[18] To determine whether the conversion of LDL to a more electronegative form by SIN-1 was associated with a decrease in reactive primary amino groups on the protein, samples were incubated with 1 mM SIN-1 for 16 h and the primary amino group content measured after reaction with TNBS.[18] It was found that free amino groups decreased from 808 ± 70 nmol/mg LDL to 690 ± 8 nmol/mg LDL after incubation with SIN-1 showing that reaction with amino groups had occurred. LDL samples treated with 1 mM SIN-1 for 16 h were found to have increased their TBARS content from a basal level of 1.2 ± 0.06 to 6.75 ± 1.29 nmol/mg protein (mean ± SD, $n = 3$), again indicating that lipid peroxidation had occurred. To confirm the hypothesis that SIN-1 initiates lipid peroxidation in the LDL particle we measured the rate of depletion of vitamin E present in the LDL particle and the concomitant formation of lipid peroxides for the first 60 min of incubation with the compound. The results are shown in Figure 3 and demonstrate that loss of vitamin E occurs rapidly on exposure to SIN-1. The rate of peroxide formation is low during this period compared to the rapid increase observed after 60 min (Figure 2B).

The effect of Antioxidants on the Oxidation of LDL

To characterise the oxidant produced by SIN-1 during incubation with LDL we have examined the effects of a number of antioxidants and antioxidant enzymes on the modification of the protein. These results are summarised in Table II. We found that SOD was able to inhibit substantially both the increase in electrophoretic mobility and formation of lipid peroxides. Since SOD is a protein, present in these experiments at a concentration of 7 μg/ml, it is possible that it could act by non-specific scavenging of the oxidant generated by SIN-1 independent of its enzymatic action. This is however unlikely since SOD that had been substantially inactivated by boiling was found to be much less effective in inhibiting the oxidative changes initiated in LDL

TABLE II
Effect of anitoxidants and antioxidant enzymes on the SIN-1-dependent modification of LDL

Treatment	R.E.M.	ROOH (nmol/mg)
None	1.13 ± 0.06	22 ± 3.82
SIN-1 (prep A)	2.44 ± 0.06	221 ± 14
SIN-1 (prep B)	2.24 ± 0.11	275 ± 16
SIN-1 (prep) C	2.68 ± 0.02	209 ± 7
SIN-1 + SOD (20 U/ml)	1.29 ± 0.07	40 ± 25
SIN-1 + boiled SOD	2.18 ± 0.06	148 ± 18
SIN-1 + Cat (500 U/ml)	2.41 ± 0.08	239 ± 49
Mannitol (100 mM)	2.5 ± 0.08	n.d.
EtOH (174 mM)	2.56 ± 0.11	271 ± 25
BHT (50 μM)	1.17 ± 0.02	20 ± 5

LDL at a concentration of 200 μg/ml isolated from donors A, B, and C was incubated for a period of 18 h at 37°C under the conditions shows. LDL isolated from donor A was used in the experiments reported in this table unless stated otherwise. When present SIN-1 was added as an aqueous solution prepared immediately before the experiment to give a final concentration of 1 mM. After this time samples were subjected to agarose gel electrophoresis and their electrophoretic mobility measured relative to an untreated sample of the same batch of LDL and the lipid peroxide content determined as described in Ref. 17. BHT was added as an ethanolic solution (1%, v/v). The activity of SOD in the boiled sample was 0.6 U/ml. Results are reported as the mean ± S.D. for a minimum of three separate experiments, n.d. = not determined.

by SIN-1 (Table II). These results suggest that superoxide plays a key role in the oxidative process. Catalase, which will metabolise any hydrogen peroxide formed from the dismutation of superoxide, had no effect on the SIN-1-induced peroxidation of LDL, demonstrating that hydrogen peroxide does not play a large role in the peroxidation process. The hydroxyl radical scavengers mannitol and ethanol did not inhibit either peroxide formation or the increase in electrophoretic mobility. In contrast, the peroxyl radical scavenger butylated hydroxy toluene (BHT) was effective in preventing both the formation of lipid peroxides and the conversion of the LDL particle to a more negative form. This latter result confirms our previous experiments which suggest that the peroxidation of LDL initiated by SIN-1 is propagated by the peroxyl radical.

Incubation of Sodium Nitroprusside(SNP) and S-nitroso-n-acetyl Penicillamine(SNAP) with LDL

Both SNP and SNAP are known to produce NO during their decomposition but unlike SIN-1 are not thought to generate superoxide.[12,13] Both compounds were incubated with LDL for 16 h and the electrophoretic mobility of the lipoprotein determined. The results of this experiment are reported in Table III in comparison with the effects of SIN-1 and show that neither SNP nor SNAP were able to modify LDL significantly. To test for the possibility that either compound may act as a peroxyl radical scavenger and so prevent the oxidation of LDL each was incubated with a sample of LDL in which peroxidation had been initiated by copper. As can be seen from Table III this transition metal is capable of causing extensive modification of LDL through the decomposition of peroxides endogenous to the LDL molecule

TABLE III
The effect of the nitic oxide generators SIN-1, SNAP and SNP on the oxidation of LDL in the presence and absence of copper

Additions	Electrophoretic mobility (REM)
None	1
1 mM SIN-1	2.44 ± 0.06
2 mM SNP	1.24 ± 0.04
1 mM SNAP	1.02 ± 0.04
Cu^{2+}	4.39 ± 0.04
2 mM SNP + Cu^{2+}	4.34 ± 0.03
Cu^{2+}	3.92 ± 0.03
1 mM SNAP + Cu^{2+}	3.72 ± 0.03

LDL (250 μg/ml) was incubated in PBS containing DTPA (100 μM) at 37°C for 18 hours under the conditions shown. Copper, when present, was at a concentration of 200 μM and the two sets of values reported above represent experiments in two different preparations of LDL. They are compared with the identical sample oxidised with Cu^{2+} in the presence of SNP or SNAP. After treatment the electrophoretic mobility of the samples was determined and is expressed relative to native (untreated) LDL. Under these conditions native LDL migrated 4.5–5 mm. Results are reported as the mean ± s.d. for three experiments.

and the initiation of lipid peroxidation.[23] Neither compound was able to act as an antioxidant in this system. It follows therefore that exposure of LDL to nitric oxide alone is unlikely to lead to extensive oxidative modification of the apoB protein.

DISCUSSION

A number of lines of evidence suggest that the oxidation of LDL occurs in the artery wall and results in a form of LDL capable of binding to the macrophage scavenger receptor.[2,3] Uptake of LDL through scavenger receptors is not down regulated and can lead to the formation of lipid-laden or "foam" cells. Such cells are associated with early atherosclerotic lesions and are considered by many to play a key role in the development of atherosclerosis.[24–26] In support of this hypothesis it has been shown that in animals treated with chain-breaking antioxidants such as probucol or BHT a lower frequency of atherosclerotic lesions occurs.[27,28] Although it is clear that oxidation of LDL occurs in the artery wall the mechanism of initiation is unknown. Several investigators, largely on the basis of experiments conducted with cultured cell systems, have suggested that a number of oxidants may be involved including superoxide and the products of lipoxygenase enzymes.[5,29–31] It is likely that, *in vivo*, a number of cell types may be involved in initiating lipid peroxidation and it follows, therefore, that a number of oxidising species could be implicated. Indeed, at different stages in the development of the atherosclerotic lesion the cellular composition and the metabolic state of the cells in the artery wall shows considerable variation raising the possibility that the oxidants contributing to oxidative stress may show a corresponding diversity. Since oxidation of LDL is likely to be involved in the initial stages of the development of the atherosclerotic lesion it is necessary to consider a source of oxidants which is endogenous to the artery wall and not dependent on the presence of inflammatory cells such as macrophages. In the present study we have investigated the possibility

that the simultaneous generation of superoxide and nitric oxide is capable of initiating lipid peroxidation. As a source of these two radicals we have used the sydnonimine, SIN-1, which we have shown previously to be capable of producing hydroxyl radicals.[11] It is most likely that the generation of this potent oxidant proceeds through the intermediate formation of peroxynitrite, the product of the reaction of superoxide and nitric oxide, which has been shown to decompose to form the hydroxyl radical.[8-10] Both superoxide and nitric oxide are produced by cells present in the normal artery wall and as such are potential candidates for the oxidants capable of promoting the oxidation of LDL in an early atherosclerotic lesion. It has already been shown that peroxynitrite is capable of initiating lipid peroxidation in pure lipid systems.[9] In support of the hypothesis that peroxynitrite may preferentially be formed from the reaction of superoxide and nitric oxide when each is produced separately we have shown that neither the reaction of nitric oxide with oxygen nor the dismutation of superoxide competes effectively with the reaction of superoxide and nitric oxide. The efficiency of the subsequent decomposition reaction of peroxynitrite to form the hydroxyl radical under physiological conditions is a matter for debate but is possibly in the region of 5–10%.[11] However, the possibility that peroxynitrite may diffuse into a lipid bilayer and subsequently decompose to form a hydroxyl radical makes it a potential candidate for initiating the abstraction of an alkyl hydrogen atom from an unsaturated fatty acid. Indeed we have found that SIN-1 is able to initiate lipid peroxidation in isolated human LDL as shown by (1) its ability to deplete the endogenous vitamin E present in LDL with the concomitant formation of lipid hydroperoxides and TBARS, (2) the loss of primary amino groups from the protein accompanied by an increase in electrophoretic mobility, and (3) the complete inhibition of this reaction by the chain-breaking antioxidant BHT.

Consistent with the hypothesis that a product of superoxide and nitric oxide is responsible for initiating lipid peroxiation we have found that the enzyme SOD is capable of inhibiting lipid peroxidation in LDL. Since the amount of nitric oxide present in solution during the autooxidation of SIN-1 is substantially increased by the addition of SOD we also conclude that nitric oxide alone is incapable of initiating lipid peroxidation.[14] This result was confirmed by incubating LDL with the nitrovasodilators SNP and SNAP which produce nitric oxide but no superoxide and had little or no ability to oxidise LDL.

Inhibition of the peroxidation reaction by SOD would also be consistent with the hypothesis that the superoxide radical itself was the principle oxidant involved in the peroxidation of LDL; however, during the autooxidation of SIN-1 little or no hydrogen peroxide is formed. This result shows that the reaction of superoxide with nitric oxide is favoured over dismutation. It follows, therefore, that the steady state concentration of superoxide in these experiments is very low and it is extremely unlikely that sufficient would be available to initiate peroxidation in LDL. In addition it has been shown that the superoxide radical is a poor initiator of lipid peroxidation unless in its protonated form, the perhydroxyl radical.[32,33] The rate of reaction for abstraction of a bis-allylic hydrogen atom from linoleic acid by the perhydroxyl radical is in the order of $10^3\ M^{-1}\ s^{-1}$ and this reaction will not compete effectively with the dismutation of superoxide or with the reaction of superoxide with nitric oxide.[33] An alternative reaction which has recently been suggested involves the reaction of the perhydroxyl radical with lipid peroxides and is possible since peroxides are present in our LDL preparations.[33] However, the reaction is extremely inefficient at neutral pH and is also unlikely to compete effectively with the reaction of superoxide and nitric

oxide.[33] We conclude therefore that the superoxide radical is not directly involved in the initiation of LDL peroxidation during the autooxidation of SIN-1.

The fact that catalase was not capable of inhibiting SIN-1-dependent lipid peroxidation and that peroxidation proceeded in the presence of the metal chelator DTPA shows that an oxidant derived from the Fenton reaction, which requires the presence of both hydrogen peroxide and transition metals, was not involved.

The hydroxyl radical scavengers mannitol and ethanol were ineffective in inhibiting the peroxidation of LDL which could be taken as evidence that hydroxyl radicals were not involved in initiating lipid peroxidation. However, other possibilities are (1) the product of mannitol or ethanol and hydroxyl radicals themselves initiate lipid peroxidation, or (2) the peroxynitrite molecule diffuses into the LDL molecule and decomposes in the lipid environment. In this latter circumstance it is unlikely that effective scavenging of the hydroxyl radical can occur.

In summary, we have shown that the autooxidation of SIN-1 releases the radicals nitric oxide and superoxide, which rapidly combine together forming an oxidant which is able to initiate peroxidation of the LDL particle. We have been unable to determine whether this oxidant is the direct product of the reaction of superoxide and nitric oxide, peroxynitrite, or one of its decomposition products, the hydroxyl radical. In a previous study we have shown that the hydroxyl radical can be formed during the autooxidation of SIN-1 so it would appear that either oxidant could play a role.[12] It is not known what the rates of nitric oxide and superoxide generation might be within the artery wall in either normal or atherosclerotic tissue. In this study we have focussed on illustrating our principal finding that the generation of superoxide and nitric oxide results in the oxidation of LDL. We have used concentrations of SIN-1 which generates these two radicals at rates considerably higher than those likely to be encountered *in vivo*. However, it is clear from earlier studies with isolated aortic rings that superoxide antagonises the action of EDRF (nitric oxide) and more recent work suggests that a similar reaction may occur *in vivo* and give rise to hypertension in the spontaneously hypertensive rat.[34,35] It is reasonable to assume therefore that a similar reaction between nitric oxide and superoxide may occur in the artery wall and lead to oxidation of lipid. Even if the reaction proceeds slowly over some days it is likely that damage to LDL will be cumulative and could contribute to oxidative modification of the LDL particle in the artery wall.

Acknowledgements

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