Nitric Oxide in Atherosclerosis

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Abstract: ·NO is produced endogenously from L-arginine by NOSs. Among its multiple activities, the homeostatic control of the vascular endothelium is crucial for atherosclerosis, a pathogenic condition connected with elevated levels of LDL, the main plasma cholesterol carrier. Oxidised LDL is proatheromatic, and toxic peroxidation products contribute to further endothelial damage. ·NO controls vascular tone, inhibits LDL oxidation and has hypocholesterolaemic activity. This review is referred to the chemistry, biology and role of ·NO on atherosclerosis and its treatment by ·NO donors or modulators.

1. NITRIC OXIDE

1.1 Biosynthesis

Nitric oxide (NO, nitrogen monoxide) is a diatomic, paramagnetic and lipophilic radical, endogenously produced via the catalytic action of nitric oxide synthases (NOSs), haem thiolate proteins which use NADPH and dioxygen to convert a guanidine nitrogen of L-arginine (L-arg) to ·NO with the formation of L-citroulline (L-citr) (Fig. **1**).

Up to now, three types of NOS have been characterised in mammals. Two isoforms are constitutively expressed and their activity depends on intracellular Ca^{2+} concentration: One is a 135 kDa enzyme in endothelial cells (eNOS or NOS III), having a N-myristoylation site that causes its localisation to the cell membrane. A second, soluble, 168 kDa protein (nNOS or NOS I) is found in neuronal and other cell types, such as kidney macula densa, β-pancreatic cells and epithelial cells of lung, stomach and uterus [1]. The third, 130 kDa inducible isoform (iNOS or NOS II) is regulated at the transcriptional level by immunological stimuli in all nucleated mammalian cells. All isoforms require calmodulin for activity, however, only iNOS contains calmodulin bound tightly even at the low, basal levels of Ca^{2+} encountered in resting cells, thus, iNOS produces a sufficient and continuous flux of ·NO until the substrates become saturated.

NOSs contain four prosthetic groups, flavin adenine dinucleotide (FAD), flavin adenine mononucleotide (FMN), iron protoporphyrin IX (haem) and tetrahydrobiopterin (BH_4). They are multimeric enzymes consisting of two identical subunits [2]. Calmodulin binding causes a rapid opening of the gate to electron flux from the reductase component of each subunit, and electrons traverse from the reduced flavins to haem, which constitutes the oxygenase component, together with the binding sites for substrates and BH_4 [3], thus initiating ·NO synthesis.

It seems that NOSs can catalyse two kinds of reactions. The first is a two-step monooxygenation reaction, converting L-arg into ·NO and L-citr. In the first step, L-arg is $N^{\hat{}}$-hydroxylated to produce the stable intermediate N^G-hydroxy-L-arg. The second step leads to the formation of nitric oxide and L-citr. In the overall reaction, it appears that NOSs transform substrates with even numbers of electrons (L-arginine, dioxygen and NADPH) into products again with even number of electrons (L-citroulline, water and $NADP^+$), plus the free radical product, ·NO. The formation of ·NO requires cooxidation of 1.5 equivalents of NADPH, that provide three electrons to the substrate. However, since NADPH can transfer electrons only two at a time, NOSs must have a means of storing the fourth, extra electron for use in the next catalytic cycle. Indeed, the flavins of NOSs, and possibly BH_4 [4, 5], make these enzymes well suited to store electrons. In this way, over the course of two catalytic cycles, three molecules of NADPH would transfer six electrons to NOS, that would be used to reduce the four dioxygen molecules consumed in the oxidation of two arginine to two nitric oxide and two citrulline molecules (Fig. **1**).

The second kind of reaction catalysed by NOSs is the formation of reduced oxygen species such as superoxide anion radical and hydrogen peroxide. In the presence of calmodulin, when either L-arg or BH_4 is insufficient, NOS utilises dioxygen as a terminal electron acceptor, and consumes NADPH at a rate that is uncoupled from ·NO synthesis. The formed $O_2^{\bullet-}$ can in turn react with ·NO, to yield higher nitrogen oxides that are extremely reactive and potentially cytotoxic. The NOS-catalysed formation of $O_2^{\bullet-}$ has been suggested to occur *in vivo* [6] and is considered to be implicated in ·NO toxicity, mostly due to the generation of peroxynitrite. Therefore, the function of NOS as an oxidase rather than a monooxygenase may contribute to severe tissue damage. In this context, there is evidence to support that BH_4 has a crucial role in the regulation of the monooxygenase versus oxidase activity of NOS [2, 7-9].

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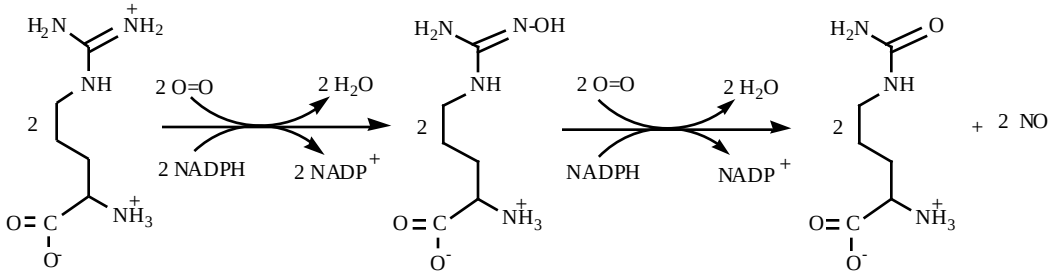

Fig. (1). Biosynthesis of nitric oxide.

1.2 Function

There is a wide array of functions mediated by ·NO in a specific manner. In the vascular endothelium, ·NO regulates blood pressure and flow. Released from the neurons in the central nervous system, ·NO affects neurotransmission and participates in learning ability and memory formation. In the periphery, several gastrointestinal, genitourinary and respiratory functions are NO-dependent. ·NO participates in host defence and immune reactions [10].

This simple molecule can influence gene transcription and mRNA translation, or produce post-translational modifications of proteins. ·NO is also able to destroy parasites and tumour cells, by direct interaction with their DNA or by inhibition of iron-containing enzymes [11].

Perhaps the most studied mechanism of the actions of ·NO is the interaction with the soluble guanylyl cyclase (GC) resulting in an intracellular elevation of cyclic guanosine monophosphate (cGMP). Guanylyl cyclase [12] occurs in a membrane-bound (particulate) and a soluble form. The cytosolic enzyme is a heterodimer of two subunits (α and β), which contain sequences similar to those at the catalytic site of the particulate form. However, the most important characteristic of the soluble GC is a haem-binding domain in the N-terminal region, which is the target of ·NO. In the absence of exogenous haem ligands, the enzyme exists at a pentacoordinate, high spin Fe^{II} state, with histidine (His105 in the , subunit) as the axial, fifth ligand. Binding of ·NO to the haem results in a pentacoordinate nitrosyl complex, where the bond to the proximal ligand is broken. This dissociation of the proximal ligand causes enzyme activation and the conversion of GTP to cGMP, performed at the catalytic domain located at the C-terminal of each subunit [10, 13].

Endothelium, lining the inner surface of blood vessels, between circulating blood and smooth musculature, responds to mechanical (e.g. haemodynamic forces) and chemical (e.g. catecholamines) stimuli by the production of ·NO, among other bioactive substances. The produced ·NO, possessing highly lipophilic character, diffuses rapidly within the vascular smooth muscle cells and causes vasorelaxation, primarily by increasing cGMP, but also via non-cGMP mechanisms, such as activation of vascular calcium-dependent potassium channels [14].

The high diffusion capacity of ·NO also contributes to the termination of its action. Leaving the cell, ·NO is inactivated in the circulation by oxyhaemoglobin, being oxidised to inorganic nitrite and nitrate.

1.3 Chemical Properties and Toxicity of Nitric Oxide

Nitric oxide is a nitrogen-centred radical with an N-O bond length (1.15 Å) intermediate between an NO triple bond (1.06 Å) and a typical double bond (ca. 1.20 Å).

As a radical, ·NO can undergo one-electron oxidation (to nitrosonium) [reaction 1], or one-electron reduction (to nitroxyl anion) [reaction 2].

$$\bullet NO \longrightarrow {}^{+}NO + e \qquad [1]$$

$$\bullet NO + e \longrightarrow {}^{-}NO \qquad [2]$$

Reaction 2 acquires a biological significance, since it can occur in cytosol, catalysed by Cu, Zn-superoxide dismutase (SOD) [15], or in mitochondria, by interaction of ·NO with the respiratory chain ubiquinol [16].

Due to its Lewis acid character, ·NO can react with a nucleophile (Nu^-) in a 2:1 ratio [reaction 3], giving diazeniumdiolate derivatives:

$$2 \cdot NO + Nu^- \longrightarrow Nu-N(O^-)-N=O \qquad [3]$$

Nitric oxide reacts with oxygen ($k=7\times10^6 M^{-1}s^{-1}$) [17], giving nitrogen dioxide [reaction 4], a potent and toxic oxidant:

$$2 \bullet NO + O_2 \longrightarrow 2NO_2 \qquad [4]$$

The final product of ·NO oxidation in an aqueous medium is nitrite [reactions 5, 6]:

$$\bullet NO + NO_2 \longrightarrow N_2O_3 \qquad [5]$$

$$N_2O_3 + H_2O \longrightarrow 2NO_2^- + 2H^+ \qquad [6]$$

NO reacts readily with superoxide anion radical ($k=6.7\times10^9 M^{-1}s^{-1}$) [18], forming peroxynitrite [reaction 7]:

$$\bullet NO + O_2^{\bullet -} \longrightarrow ONOO^- \qquad [7]$$

Superoxide anion radical is considered to occur *in vivo* under conditions of oxidative stress (such as inflammation or reoxygenation of a tissue after ischemia or hypoxia) and is one of the main targets for ·NO in the cellular environment [19].

Peroxynitrite has almost neutral pKa, and is a species considerably stable in alkaline solutions (pH 12-13). However, the acid form, peroxynitrous acid, is very unstable (with a half life of about 1 second at pH 7.0) [20], and finally yields nitric acid [reaction 8]:

$$ONOO^- + H^+ \rightleftharpoons ONOOH \longrightarrow \left[HO^\bullet + NO_2^\bullet\right] \longrightarrow HNO_3 \quad [8]$$

It has been claimed that peroxynitrous acid may generate hydroxyl radical and $NO_2^\bullet$ [reaction 8] via homolytic cleavage of the O-O bond [21, 22]. However, several arguments have been reported lately that homolysis of peroxynitrous acid is not favoured thermodynamically [23], and an isomerisation of peroxynitrous to nitric acid is more likely to occur.

Peroxynitrite can cause nitration, hydroxylation and oxidation, the main targets in biological systems being lipids, proteins and nucleic acids [24]. Thus, the toxicity of ·NO is greatly due to peroxynitrite formation. In pathological conditions connected with high ·NO generation, e.g. systemic hypotension or cytokine mediated lung injury [25], peroxynitrite is formed in high steady-state conditions. Normally, tissue ·NO concentration is low, but, during acute events, e.g. inflammation, it may reach a maximum of about 1 μM. Therefore, it may then become cytotoxic.

1.4 Detection and Determination of Nitric Oxide

The detection of ·NO, either produced endogenously in biological systems or liberated from exogenous nitric oxide donors, agents which are able to release ·NO spontaneously or after activation, presents several difficulties, mainly because it is unstable when dissolved in buffer solutions. However, a number of direct or indirect methods have been developed for the determination of ·NO.

Direct methods involve spin trapping reactions, followed by EPR spectroscopy [26, 27]. Another method is the oxidation of oxyhaemoglobin to methaemoglobin by ·NO giving nitrate [28].

Indirect techniques are based on the determination of nitrite and nitrate as the oxidation products of ·NO. Among them, the most widely used is the colourimetric determination of the azo dye formed by applying the Griess reaction: nitrite, in acidic conditions, reacts with sulphanilamide to the diazonium salt, which in turn is coupled to N-(1-naphthyl)ethylene diamine, yielding a red azo derivative with an absorption maximum at 540nm [29]. However, since the ratio of nitrite and nitrate in biological fluids or culture media varies substantially, nitrate should be reduced to nitrite prior to the determination with the Griess reagent [30]. For this conversion, nitrate reductase can be used for samples without high protein content, or cadmium as suitable means for inexpensive and rapid quantification of total nitric oxide.

Finally, efficient fluorometric assays have been developed and reported to be several times more sensitive than the Griess colourimetric determination [31-33].

2. ATHEROSCLEROSIS

2.1 General

Atherosclerosis is a multifactorial disorder, the main cause of morbidity and mortality in the western societies. Many forms of cardiovascular disease are associated with atherosclerosis, a local thickening of the arteries. According to the World Health Organisation, atherosclerosis is "a disease of the intima of the arteries involving focal accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissue and calcium deposits, and associated medial changes" [34].

In the affected artery, the pathologically recognised, progressive atherosclerotic lesions, which differ mainly in the predominant morphological distribution and in the chemical nature of the accumulated lipids, are foam cells, fatty streaks and fibrous plaques (or atheroma).

Foam cells are deformed cells laden with lipids. They form the *fatty streaks*, considered to be the precursors of *fibrous plaques*, which typically are characterised by a fibrous cap and a deeper necrotic core. Fibrous (or atheromatic) plaques can gradually obstruct the arterial lumen, leading to thrombosis.

The major change in the atheromatous artery is the significant elevation of cholesterol content, accompanied by changes in the particular fatty acids of the cholesterol esters. In the intracellular lipids of the fatty streak, cholesterol is esterified mostly with oleic acid, while, in the extracellular lipid of the plaque, cholesterol esters of linoleic acid are the most abundant. The lipids of the plasma represent the fatty acid pattern of cholesterol esters of the plaque, but not of the fatty streak.

It is known [35] that the greatest amount of blood cholesterol is associated to plasma constituents, the various lipoprotein fractions. These are stable complexes of lipid, protein and carbohydrate, and are classified into four groups (Chylomicron, Very Low Density Lipoprotein, Low Density Lipoprotein and High Density Lipoprotein), according to their electrophoretic mobility and density. The electrophoretic mobility of the lipoproteins characterise their protein component, or apoprotein, whereas an increase in density means a decrease of their lipid content.

High Density Lipoprotein (HDL) is exceptionally rich in protein and is involved mainly in the mobilisation of cholesterol from the tissues.

In humans, Low Density Lipoprotein (LDL) forms the greatest part of all lipoproteins and is particularly rich in cholesterol and its esters, cholesteryl linoleate being the

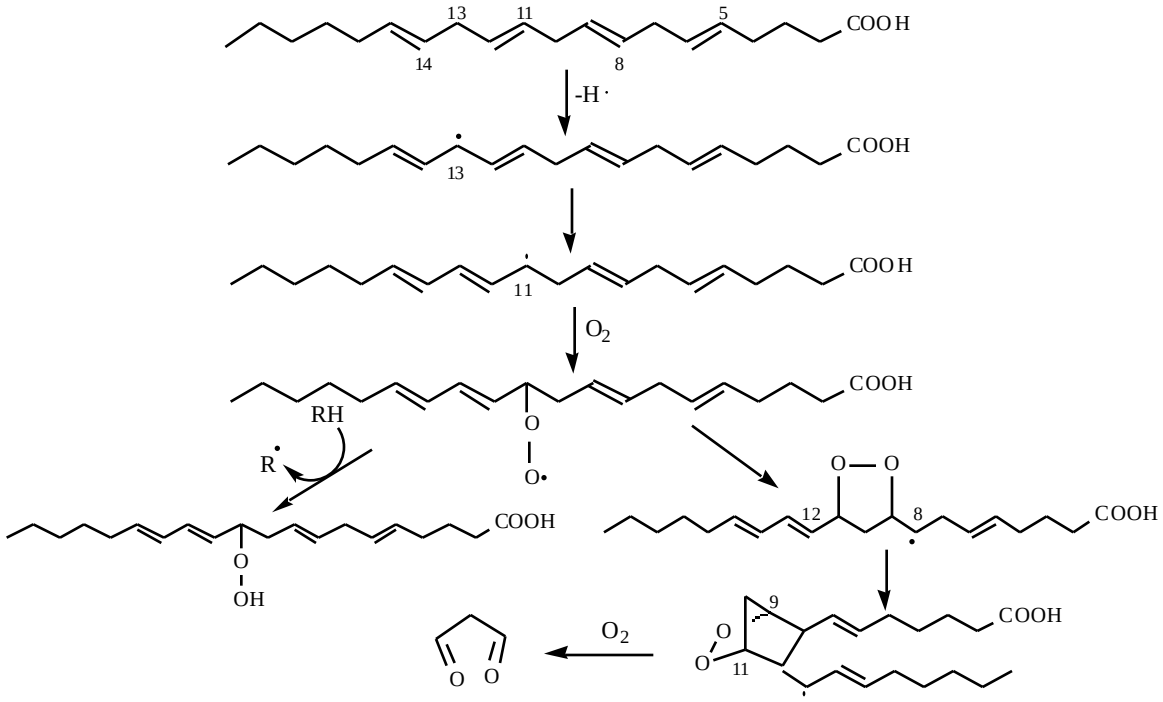

Fig. (2). Chain reactions of lipid peroxidation.

principal oxidizable lipid. The LDL particle contains a core of cholesteryl esters and triglycerides, surrounded by a monolayer of phospholipids, unesterified cholesterol and one molecule of apolipoprotein B-100 (apo B). LDL constitutes the main cholesterol carrier in the plasma and is intimately connected with the aetiology and progression of atherosclerosis, since elevation in LDL plasma levels is sufficient to induce the atherosclerotic reaction [36].

There is convincing evidence that nitric oxide can interfere with the metabolism of lipoproteins. It has been found that treatment of hypercholesterolaemic experimental animals with NOS inhibitors promoted atherosclerosis [37], whereas the administration of ·NO donors produced a dose-dependent decrease of cholesterol level and atherosclerosis [38], probably by counteracting the depletion of ·NO caused by hypercholesterolaemia. It has also been reported that the ·NO donor S-nitroso-N-acetylpenicillamine suppressed net apoprotein B production in HepG2 cells via an apo B-specific, cellular sterol-independent mechanism [39].

2.2 Reactive Oxygen Species and Atherosclerosis

Phospholipids and cholesteryl esters containing polyunsaturated fatty acids (PUFA) are primary targets of reactive oxygen species, due to the easily abstractable bis-allylic hydrogen atoms. Oxygen derived free radicals, formed by the one-electron reduction of molecular oxygen, may attack PUFA resulting to the formation of a carbon-centred lipid radical. The latter can spontaneously react with molecular oxygen to form a lipid peroxyl radical (LOO·), thus promoting the chain reaction of lipid peroxidation: A peroxyl radical can abstract a hydrogen atom from another PUFA, or form a lipid hydroperoxide or cyclic peroxide. Additionally, carbon-centred lipid radicals may form conjugated dienes with an absorbance maximum at 234nm (Fig. **2**).

Oxygen derived free radicals may also attack proteins and nucleic acids. Membrane cholesterol may be another target of oxidative insult (Fig. **3**), since it has been found that cholesterol included in liposomes can be peroxidised, mainly yielding cholesterol-7-hydroperoxides, epoxides and cholestanediols [40a].

The non-radical products of radical chain reactions may accumulate or react with other species, e.g. malondialdehyde (Fig. **2**) and 4-hydroxynonenal may react with lysine residues leading to covalent modification of proteins, via the intermediate Schiff base. They may also be substrates for further enzymatic processes, e.g. glutathione disulphide (GSSG) reduction back to glutathione (GSH) by glutathione reductase.

Crucial components of chain reactions in biological systems are dioxygen and transition metal ions. The potential toxicity of free radicals depends on their site of production, the biological relevance of their (intracellular) target, their physical and chemical characteristics (e.g. polarity, reactivity) and the level of the antioxidant defence mechanisms of the organism [41]. An imbalance in the organism favouring the pathways leading to the accumulation rather than the removal of free radicals is known as *oxidative stress*.

The origin of atherosclerosis lies in an injury, often a radical attack to the vascular endothelium, since endothelial dysfunction appears before the development of atherosclerosis in several associated conditions, such as

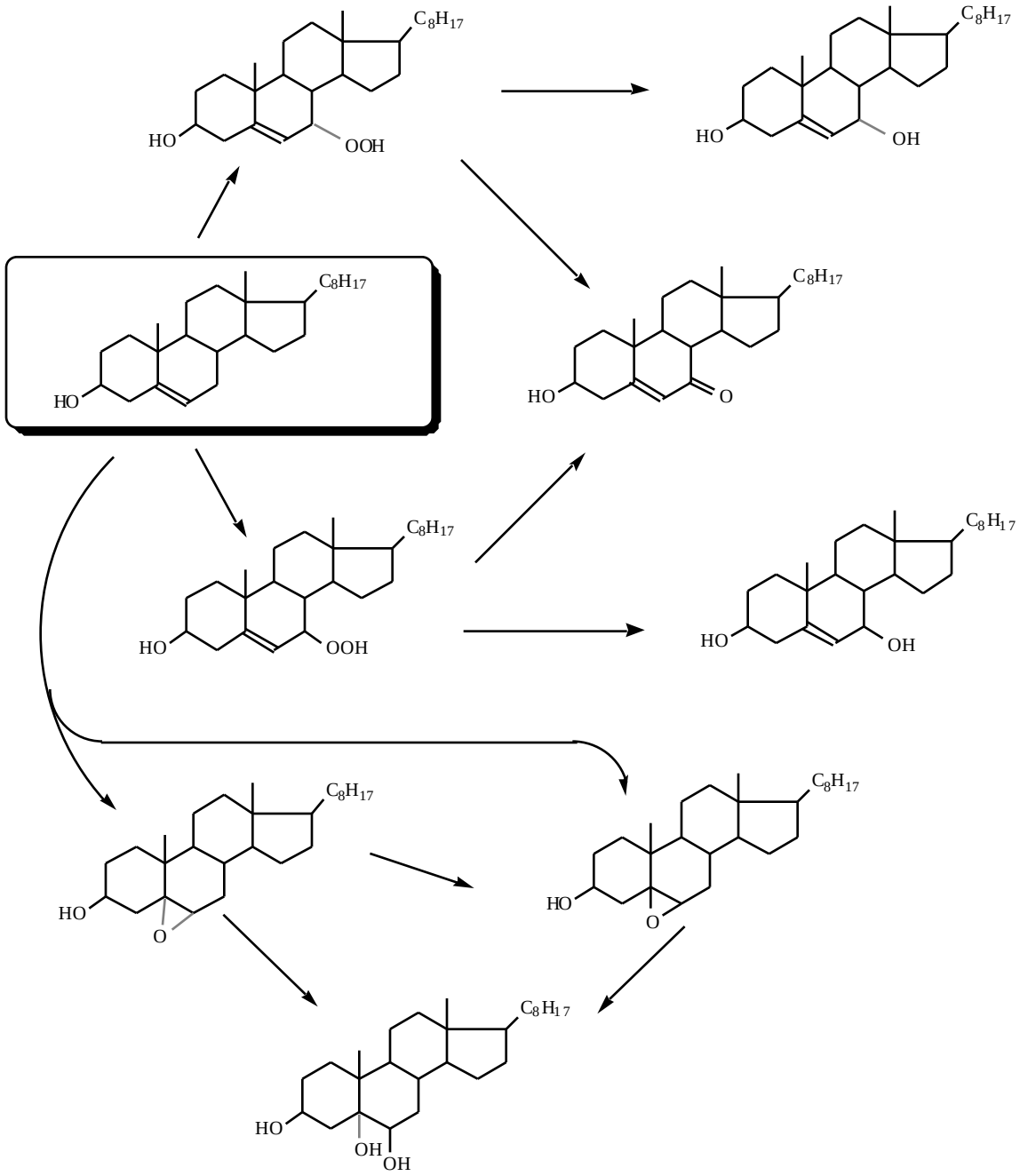

Fig. (3). Cholesterol peroxidation.

diabetes mellitus, hypertension and hypercholesterolaemia. Radicals may initiate the formation of macrophages within the vessel wall, which generate $O_2^{\bullet -}$ and yield hydrogen peroxide and peroxynitrite. Macrophages also cause the release of hydrolytic enzymes and of agents inducing smooth cell proliferation, leading to further injury of the surrounding cells [42, 43]. Arterial endothelial cells, smooth muscle cells and macrophages are able to oxidise LDL [40b] through multiple mechanisms, and the generation of peroxidation products may in turn aggravate the initial endothelial damage.

It is unanimously accepted today that there is a clear cause and effect relationship between oxidation of LDL and the pathogenesis of atherosclerosis. Atheromatic lesions contain a number of oxidised lipids derived from those in LDL. Oxidised LDL (ox-LDL) is not recognised by the LDL receptor, but it has a high affinity to the macrophage scavenger receptor, resulting to an uncontrolled uptake of the lipoprotein in the vessel wall and the generation of foam cells [44-46]. Furthermore, ox-LDL is a potent chemo-attractor for blood monocytes into the atheromatic lesion, and an inducer of eicosanoid production. Ox-LDL can promote the expression of endothelin and inhibit the expression of NOS [36]. Lysophosphatidylcholine, a product of LDL oxidation, is chemoattractive for monocytes and T-lymphocytes and induces adhesion molecules.

2.3 Reactive Oxygen Species and Nitric Oxide

Activation of oxygen is a physiologic process in aerobic life and the organisms have developed mechanisms of protection against radical injury, such as antioxidant enzymes (SOD, catalase, glutathione peroxidase), other proteins (transferrin, albumin) or low molecular weight substances, lipophilic (·-tocopherol, ,-carotene) or water-soluble (glutathione, ascorbic acid). Since ·NO participates in the general homeostatic control of the vasculature, it is expected to play a key role in the regulation of the antioxidant balance of the organism. Although the reaction of ·NO with $O_2^{\bullet-}$ yields the toxic peroxynitrite, ·NO can also directly inhibit superoxide-produced cell injury, terminate lipid peroxyl (LOO·) and oxyl (LO·) radical chain reactions and reduce LDL oxidation [47]. Consequently, the pro-oxidant versus antioxidant activity of ·NO depends highly on the relative concentrations of the individual reactants in the microenvironment of the ·NO generation and the local flux of $O_2^{\bullet-}$. At low concentration, ·NO has a potent inhibitory effect on the oxidative modification of LDL and protects against reactive oxygen species-associated damage, whereas high concentrations of ·NO may be cytotoxic. Thus, the level of the vascular antioxidant potential has a great impact on the endothelial ·NO bioactivity.

3. TREATMENT OF ATHEROSCLEROSIS

3.1 Current Approaches

The therapeutic approaches to atherosclerosis include reduction of plasma cholesterol and lipid levels, prevention of LDL oxidation and control of vascular tone with improvement of endothelial function.

Cholesterol lowering therapy can improve ·NO-dependent vasorelaxation in hypercholesterolaemic patients [48]. Antioxidants and $O_2^{\bullet-}$ scavengers would restrict LDL oxidation and inhibit peroxynitrite formation [49]. Improved endothelial function and ·NO activity have been demonstrated with vitamin E and ascorbic acid treatment [50, 51]. Probucol, a hypocholesterolaemic, lipid soluble compound with antioxidant properties, has been reported to limit LDL oxidation *in vitro* [52], to inhibit atherosclerosis in rabbits [53] and to restore endothelial dysfunction [54].

Treatment of atherosclerosis in a more direct connection with ·NO activity may be either control of the production and bioactivity of endogenous ·NO or administration of ·NO from exogenous sources.

3.2 Modification of Endogenous Nitric Oxide

Insufficiency of ·NO-mediated effects may be due to impaired activity and availability of NOSs, their cofactors and ·NO precursors.

Increasing the levels of L-arginine in the endothelium would be a rational way to restore vasodilator dysfunction in atherosclerosis. There is evidence that chronic L-arginine administration inhibited the progression of atheromatic plaque formation {55]. Dietary supplementation with L-arginine can also be effective on pre-existing lesions. It has been reported that, if hypercholesterolaemic rabbits with atherosclerotic lesions covering 30% of the aorta surface, begin receiving dietary L-arginine, lesion formation is inhibited or even declined [56].

Besides providing the ·NO precursor, the beneficial effect of L-arginine administration on endothelial function results from its ability to decrease $O_2^{\bullet-}$ production and activity, by directing NOS activity to monooxygenation reactions, to inhibit monocyte adhesion and to prevent thrombosis. The effect of L-arginine on monocyte adhesion is attributed to ·NO-dependent inhibition of adhesion molecules [57]. Thrombosis, resulting from platelet aggregation, is decreased mainly through elevated platelet cGMP production and inhibition of thromboxane B_2 [58].

3.3 Exogenous Nitric Oxide - Nitric Oxide Donors

Nitric oxide donors are agents of diverse chemical structures that share the common property of liberating ·NO in the organism in a form capable to produce the biological effects of endogenous ·NO, e.g. vasorelaxation. They constitute a special class of prodrugs finding clinical interest, e.g. treatment of angina, hypertension, thrombosis, or applications in biomedical research.

Common ·NO donors with low oxidation states of nitrogen, such as nitroprusside, nitrosamines and nitrosothiols, can liberate ·NO after thiol-mediated reduction [59]. Organic nitric esters, with high oxidation states of nitrogen, such as glyceryl trinitrate (nitroglycerin), isosorbide dinitrate or 5-mononitrate, erythrityl tetranitrate and nicorandil(N-[2-(nitrooxy)ethyl]-3-pyridinecarboxamide) liberate ·NO after biotransformation, most probably by multiple enzyme systems with regional selectivity [60].

One disadvantage in the chronic use of these donors is the development of nitrate resistance and tolerance.

Furthermore, the diazeniumdiolate derivatives ($NuN_2O_2^-$, NONOates, reaction 3) release ·NO spontaneously, without any redox activation. Thus, the proline derivative 1-[2-(carboxylato)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate has been found to possess antiplatelet and vasodilatory activity [61].

Other types of ·NO releasing compounds are under extensive study, some of them are presented herein.

3.4 Nitric Oxide Donors and Atherosclerosis

Most of the common ·NO donors, especially organic nitrates, have been used for years to induce ·NO dependent vasodilatation.

Nitric oxide donors have been proven useful in the treatment of atherosclerosis since, in addition to vasorelaxation that improves blood flow, they can inhibit

platelet activation [62], which promotes coronary atherosclerosis, thrombosis and ischemic events.

Nitroglycerin and sodium nitroprusside have been found to specifically inhibit platelet interaction with damaged coronary arteries [63, 64].

Systemic administration of 3-morpholinosydnonimine (SIN-1), a spontaneous ·NO donor, reduces platelet thrombus formation [65]. SIN-1 is a metabolite of molsidomine (N-carboxy-3-morpholinosydnonimine ethyl ester), a coronary vasodilator and antianginal agent with low susceptibility to nitrate tolerance.

S-nitroso-N-acetylpenicillamine, (SNAP), a widely studied nitrosothiol, has been found to inhibit macrophage-dependent oxidation of LDL [66] and to completely reverse endothelin-1 mediated constriction in human arteries [67].

Some diazeniumdiolate derivatives with different half lives (1 - 38 min) have been reported recently to inhibit cholesterol peroxidation in large unilamellar liposomes [68], one of the most potent being (*Z*)-1-{N-methyl-N-[6-(N-methylammoniohexyl)-amino]}diazen-1-ium-1,2-diolate (MANO).

Various structurally different ·NO donors, other related agents and delivery systems are also investigated in connection with ·NO-associated cardiovascular disease.

Some triazolimine derivatives have been prepared as ·NO donors and tested for antiplatelet and antithrombotic activity. Among them, 5-amino-3-phenyl-1,2,3,4-oxatriazolimine chloride and its N-ethoxycarbonyl derivative have been found respectively to inhibit platelet aggregation, and thrombus formation in arterioles after oral administration to rats, by about 50%. This effect has been attributed to the ·NO donating activity of the intermediately formed nitrosohydrazine [69].

Another 1,2,3,4-oxatriazolinium ·NO donor has been found to inhibit neutrophil adhesion and E-selectin expression in human, lipopolysaccharide-stimulated vascular endothelial cells [70].

In an analogous experimental setting, two cysteine-containing, spontaneous or after bioactivation ·NO donors are found to be potent inhibitors of endothelial activation [71].

Benzodifuroxan and benzotrifuroxan (but not benzofuroxan) are reported to display potent vasodilatating activity and to increase cytosolic cGMP levels, acting as typical ·NO donors [72].

A number of S-nitrosated dipeptides from N-acetylpenicillamine and various amino acid methyl esters with ·NO-releasing and possible tissue penetrating properties have been reported as vasodilators [73].

The antithrombotic and stroke preventing potential of atorvastatin, a statin inhibitor of 3-hydroxy-3-methylglutaryl coenzyme-A reductase, has been estimated and attributed to eNOS upregulation [74].

Finally, ·NO-releasing biomaterials have been developed by ·NO incorporation into photopolymerised polyethylene glycol hydrogels and found to inhibit smooth muscle cell proliferation and platelet adhesion [75].

In conclusion, ·NO, a simple molecule with a large variety of biological functions, is a key factor for the vascular homeostasis and of great importance in atherogenesis. Therapeutic approaches to the prevention and treatment of atherosclerosis based on ·NO bioactivity attract special interest and are becoming a challenge for future studies and further applications.

ABBREVIATIONS

apo B = Apolipoprotein B-100

BH_4 = Tetrahydrobiopterin

cGMP = Cyclic guanosine monophosphate

EPR = Electron paramagnetic resonance

GC = Guanylyl cyclase

HDL = High density lipoprotein

LDL = Low density lipoprotein

MANO = (*Z*)-1-{N-methyl-N-[6-(N-methylammoniohexyl)-amino]}diazen-1-ium-1,2-diolate

NOS = Nitric oxide synthase

ox-LDL = Oxidised low density lipoprotein

PUFA = Polyunsaturated fatty acid

SIN-1 = 3-Morpholinosydnonimine

SNAP = S-Nitroso-N-acetylpenicillamine

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