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REVIEW

Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors

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Reactive carbonyl compounds (RCCs) formed during lipid peroxidation and sugar glycoxidation, namely Advanced lipid peroxidation end products (ALEs) and Advanced Glycation end products (AGEs), accumulate with ageing and oxidative stress-related diseases, such as atherosclerosis, diabetes or neurodegenerative diseases. RCCs induce the 'carbonyl stress' characterized by the formation of adducts and cross-links on proteins, which progressively leads to impaired protein function and damages in all tissues, and pathological consequences including cell dysfunction, inflammatory response and apoptosis. The prevention of carbonyl stress involves the use of free radical scavengers and antioxidants that prevent the generation of lipid peroxidation products, but are inefficient on pre-formed RCCs. Conversely, carbonyl scavengers prevent carbonyl stress by inhibiting the formation of protein cross-links. While a large variety of AGE inhibitors has been developed, only few carbonyl scavengers have been tested on ALE-mediated effects. This review summarizes the signalling properties of ALEs and ALE-precursors, their role in the pathogenesis of oxidative stress-associated diseases, and the different agents efficient in neutralizing ALEs effects *in vitro* and *in vivo*. The generation of drugs sharing both antioxidant and carbonyl scavenger properties represents a new therapeutic challenge in the treatment of carbonyl stress-associated diseases.
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Keywords: ALE; atherosclerosis; cancer; carbonyl scavenger; carbonyl stress; diabetes; 4-hydroxynonenal; inflammation; lipid peroxidation; neurodegenerative diseases

Abbreviations: AGE, advanced glycoxidation end products; ALE, advanced lipid peroxidation end products; MDA, malondialdehyde; 4-HNE, 4-hydroxynonenal

Introduction

Reactive carbonyl compounds (RCCs) formed endogenously during lipid peroxidation and the glycoxidation of carbohydrates are precursors of advanced glycation end products (AGEs) and advanced lipid peroxidation end products (ALEs), which form cross-links on tissular proteins (carbonyl stress), and accumulate during ageing and in chronic diseases (Dalle-Donne *et al*., 2003; Smit and Lutgers, 2004). Carbonyl stress induces progressively protein dysfunctions and damages in all tissues, with pathological consequences such as inflammation and apoptosis contributing to the progression of diseases (Dalle-Donne *et al*., 2003; Petersen and Doorn, 2004). Therefore, inhibiting the chemical modification of tissue proteins may prevent the pathological consequences of carbonyl stress and may represent a new therapeutic strategy for patients. Most of the carbonyl stress inhibitors used so far have been developed to prevent the accumulation of AGEs in diabetes and its complications including accelerated atherosclerosis, nephropathy, cataract and neuropathies (Thomas *et al*., 2005; Peyroux and Sternberg, 2006). In contrast, except antioxidants that inhibit indirectly the generation of lipid peroxidation products, few carbonyl scavenger agents known to reduce *in vitro* the accumulation of ALEs precursors have been tested *in vivo* on the progression of ALE-related diseases. This review summarizes the mechanisms involved in the generation of ALE precursors, their targets and role in carbonyl stress and their consequences in ageing and in the pathogenesis of diseases. The review then focusses on carbonyl scavenger agents able to neutralize *in vitro* and *in vivo* protein modifications induced by ALE precursors, and their potential interest in pre-clinical and clinical studies, as new pharmacological approaches in carbonyl stress-related diseases.

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Formation and signalling properties of ALEs and ALE precursors

Lipid peroxidation induced by oxidants and oxidative stress, generates a huge variety of lipid peroxidation products, including RCCs and more stable products such as ketones and alkanes (Figures 1 and 2). Moreover, the α-oxoaldehyde methylglyoxal may be generated during glycoxidation (Thornalley *et al*., 1999) and as a by-product of catabolism of lipids, glucose and amino acids (Figure 3) (Peyroux and Sternberg, 2006).

RCCs such as aldehydes and dicarbonyls, including hydroxyalkenals, acrolein, malondialdehyde (MDA), glyoxal and methylglyoxal, exhibit a large panel of biological properties. These aldehydes react on cellular and tissular proteins to form adducts (ALEs) that induce protein dysfunctions and alter cellular responses (Zarkovic, 2003; Petersen and Doorn, 2004). The rate of oxidation and aldehyde adduct formation is low under physiological conditions, but increases with ageing together with the decrease in antioxidant defences (McEwen *et al*., 2005; Voss and Siems, 2006). It is a slow process countered by the rapid turnover of short half-life cellular proteins, whereas modified long-life proteins accumulate in tissues with age (Lyons *et al*., 1991).

Formation of ALE precursors or RCCs

The oxidation of polyunsaturated fatty acids (PUFAs) generates RCCs, including highly reactive α,β-unsaturated hydroxyalkenals, such as 4-hydroxynonenal (4-HNE) and 4-hydroxyhexenal (4-HHE). Oxidation of *n*-6 PUFAs (mainly linoleic and arachidonic acids) leads to the formation of 4-HNE (Esterbauer, 1993), whereas oxidation of *n*-3 PUFAs (docosahexaenoic acid, eicosapentaenoic acid and linolenic acid) generates 4-HHE (Van Kuijk *et al*., 1990).

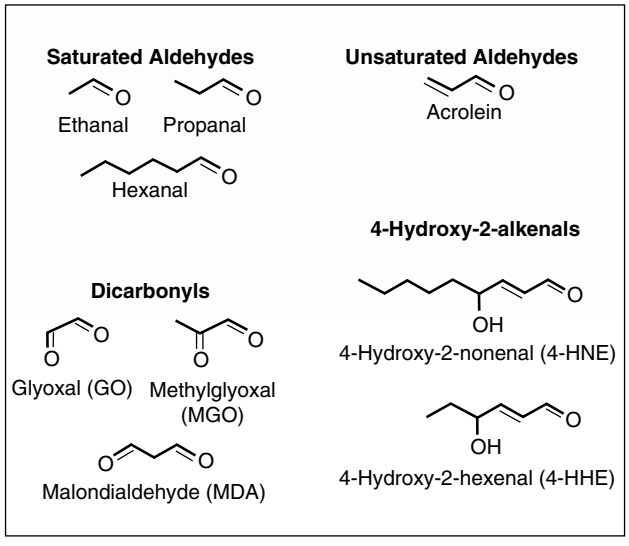

Figure 1 Chemical formulae of some aldehydic compounds derived from lipid peroxidation, including saturated aldehydes, unsaturated aldehydes, 4-hydroxy-2-alkenals and dicarbonyls. These reactive carbonyl compounds are also advanced lipid peroxidation end product precursors.

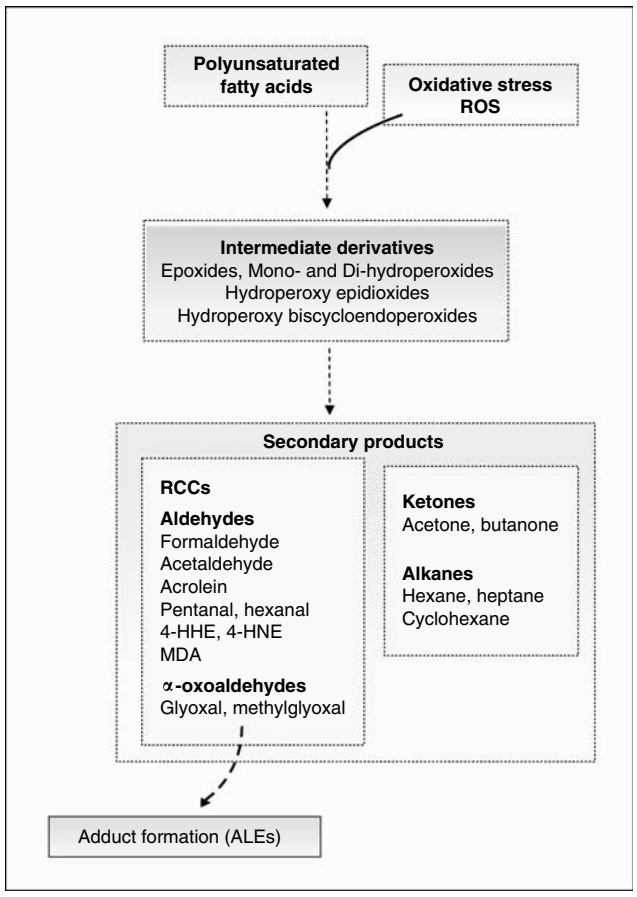

Figure 2 Schematic steps of lipid peroxidation leading to the formation of secondary products and ALEs (Uchida, 2000; Aldini *et al*., 2006; Shibamoto, 2006). RCCs are able to react with proteins and other biological molecules, thereby forming ALEs. Stable products (such as alkanes) do not react with proteins. ALEs, advanced lipid peroxidation end products; RCCs, reactive carbonyl compounds.

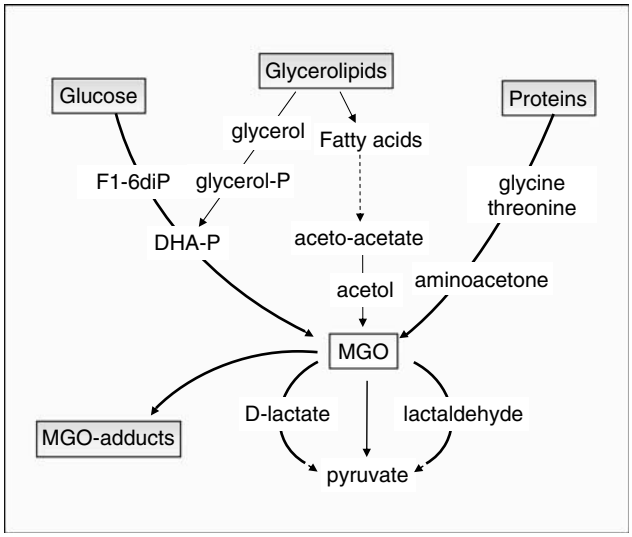

Figure 3 Metabolic pathways leading to the formation of methylglyoxal (MGO) and of MGO adducts (Ramasamy *et al*., 2006). In addition to lipid peroxidation and glycoxidation, MGO can be formed as a by-product of glucose, glycerolipids and amino acids.

4-HNE can react with histidine (His), cysteine (Cys) or lysine (Lys) residues of proteins, leading to the formation of stable Michael adducts with a hemiacetal structure (Schaur, 2003). The chemical reactions involved in 4-HNE interactions with proteins are summarized in Figure 4, and include reactions between the C = C double bond with a nucleophile (Cys, glutathione (GSH) and amine) via 1,2- and 1,4-Michael addition (Nadkarni and Sayre, 1995). The 1,2-Michael addition involves the reaction of a primary amine (Lys) with the α,β-unsaturated carbonyl, resulting in the formation of a Schiff base at acidic pH. This step is reversible (Petersen and Doorn, 2004).

The 1,4-Michael addition of 4-HNE involves the reaction of the nucleophile with the β-carbon, resulting in the addition of the nucleophile and proton across the C = C double bond (Nadkarni and Sayre, 1995). Among the protein residues that react with 4-HNE, Cys exhibits the highest reactivity, followed by His and Lys (Petersen and Doorn, 2004), but Cys–HNE adducts could be less stable than His–HNE adducts (Uchida, 2003). Concerning the modification of apolipoprotein B, 4-HNE was reported to attack mainly the Lys and to a lesser extent His and Cys residues (Jürgens *et al*., 1986).

MDA and acrolein are formed during lipid peroxidation and bind to nucleophiles (Poli and Schaur, 2000). MDA is one of the most abundant aldehydes, resulting from peroxidation of arachidonic, eicosapentaenoic and docosahexaenoic acid (Esterbauer *et al*., 1991). MDA reacts with Lys residues by forming Schiff bases (Esterbauer, 1993), and plays a major role in low-density lipoprotein (LDL) modification and their metabolic deviation towards macrophages (Palinski *et al*., 1989; Steinberg, 1997). Protein modifications by bifunctional aldehydes can also lead to intramolecular or intermolecular protein cross-linking. The accumulation of MDA adducts on proteins is involved in the formation of the fluorescent pigment lipofuscin, which accumulates progressively during ageing (Chowdhury *et al*., 2004). Acrolein (CH2 = CH–CHO) is also formed during lipid peroxidation and is a strong electrophile exhibiting high reactivity with Cys, His and Lys nucleophile residues (Uchida *et al*., 1998).

Another class of reactive ALE precursors is represented by α-oxoaldehydes (methylglyoxal and glyoxal) (Figure 1). These agents are basically generated during diabetes and hyperglycaemia, from the Maillard reaction, which involves the condensation of sugars (glucose) with proteins, leading to the formation of a Schiff base, followed by a rearrangement (non-enzymatic glycation) generating the Amadori product. During Amadori rearrangement, α-oxoaldehydes (methylglyoxal and glyoxal) are formed, and react with Lys and arginine residues to form AGEs. These α-oxoaldehydes are also generated from lipid peroxidation (glyoxal), the catabolism of ketone bodies and the fragmentation of triosephosphates (methylglyoxal) (Figure 3). α-Oxoaldehydes are increased in the early stages of glycation in hyperglycaemic conditions, and are detected in atherosclerotic lesions (Thornalley *et al*., 1999).

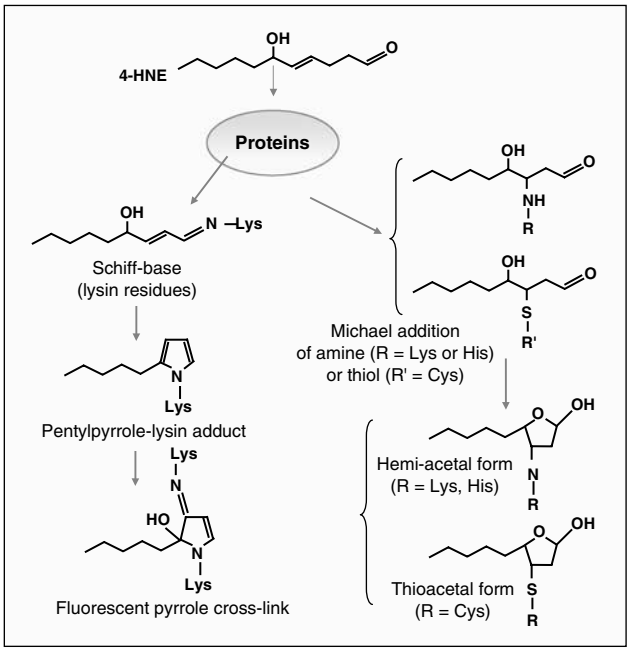

Figure 4 Schematic reactions of 4-HNE with proteins leading to the formation of 4-HNE-protein adducts. (1) Schiff base formation on primary amine (for example, lysin) leading to more complex compounds, such as pyrrole-Lys adducts and fluorescent cross-links. (2) Michael addition of 4-HNE on amino groups (Lys and His) or thiols (Cys or GSH), followed by cyclization and hemi-acetal or hemi-thioacetal formation (Sayre *et al*., 1993; Uchida 2000; Carini *et al*., 2004). 4-HNE, 4-hydroxynonenal; Lys, lysine; His, histidine; Cys, cysteine; GSH, glutathione.

Methods for the detection of ALE precursors and ALEs modified protein (or RCC/protein adducts)

Determination of ALEs precursors

Several analytical methods have been used to determine RCCs ranging from whole evaluation of RCCs by poorly specific methods, such as TBA reactive substances assay, to highly specific methods allowing a precise structural determination and quantitation.

As ALE precursors are formed in lipid fractions, a first difficulty is because RCCs are chemically reactive compounds that may react during sample preparation. It is therefore necessary to prepare RCCs derivatives, generally as dinitrophenylhydrazine (DNPH) derivatives (Esterbauer *et al*., 1991). More recently, RCCs derivatives of pentafluorophenylhydrazine, *N*-methylhydrazine, cysteamine and of *o*-phenylene diamine have been used successfully and exhibit several analytical advantages, as reviewed recently by Shibamoto (2006).

Another difficulty is the isolation and extraction of these compounds from lipid-rich samples. The classical extraction by solvent partition is difficult because of the amphiphilic character of numerous lipid peroxidation products. This led to develop new and more sophisticated methods, utilizing solid phase extraction cartridge, or 'simultaneous purging and solvent extraction apparatus' (see review by Shibamoto, 2006).

DNPH derivatives converted into their corresponding hydrazones can be separated and analysed by various methods including gas chromatography, gas chromato-

graphy/mass spectrometry, HPLC/UV (high-performance liquid chromatography/ultraviolet) and HPLC/MS (mass spectometry) (Shibamoto, 2006).

Detection of ALEs modified proteins

Classical methods to detect and evaluate carbonyl content of modified protein (ALEs modified proteins) are based on the reaction of DNPH with carbonyl groups to form DNP-hydrazone derivatives that are measured by spectrophotometry. An alternative enzyme-linked immunosorbent assay (ELISA) method is the detection of the DNP moiety of the proteins by specific antibodies (Buss *et al*., 1997).

Recently, proteomics (mass spectrometry) methods have been used to identify ALEs modified proteins (RCC/protein adducts) after proteolytic digestion and analysis of peptide mass fingerprints by MALDI-TOF-MS or LC-ESI-MS/MS (Carini *et al*., 2004; Aldini *et al*., 2006; Sayre *et al*., 2006).

Polyclonal and specific monoclonal antibodies against RCC–protein adducts (including anti-4-HNE and anti-acrolein antibodies) allowed to visualize the presence of such modified proteins in various tissues, under physiological and pathophysiological conditions (Jurgens *et al*., 1990; Uchida *et al*., 1995; Waeg *et al*., 1996; Xu *et al*., 2000). Recently, this also allowed developing ELISA techniques for quantitative determination of oxidatively modified LDL and circulating proteins (Satoh *et al*., 1999; Borovic *et al*., 2006). Moreover, modification of specific cellular or tissular protein can be evaluated by western blot of the immunoprecipitated protein revealed with specific antibodies (for example, anti-HNE and anti-CML), and the modification can be compared to functional changes (Suc *et al*., 1998; Vindis *et al*., 2006).

Molecular and cellular targets and signalling properties of ALEs and ALE precursors

ALEs precursors play an active role in signal transduction by altering progressively the structure and function of circulating and tissular proteins, with consequences on the inflammatory status, cell proliferation and viability (Petersen and Doorn, 2004). The biological effects of ALE precursors are modulated by their local concentration, their availability depending on the presence of cellular detoxifying or metabolizing systems, for instance the conjugation of 4-HNE with cellular GSH catalysed by glutathione *S*-transferase (GST) and the functionality of proteolytic systems involved in the degradation of modified proteins (Petersen and Doorn, 2004).

LDL modification

One of the best-known effects of ALEs precursors is their role in the modification of LDLs which is involved in the formation of early atherosclerotic lesions, according to the oxidative theory for atherosclerosis (Chisolm and Steinberg, 2000). LDL oxidation is a slow process, which occurs in the sub-intimal space, by contact of LDLs with reactive oxygen species (ROS) generated by vascular cells. Oxidized LDLs (oxLDLs) can be obtained *in vitro* by incubating LDLs with oxidants or cultured vascular cells (Jurgens *et al*., 1987; Steinberg, 1997). The oxidation process generates a huge variety of lipid peroxidation products among them MDA, 4-HNE, acrolein or glyoxal (Esterbauer *et al*., 1991; Esterbauer, 1993). As summarized in Figure 5, these aldehydes react with the Lys residues of apoB, which are required for LDL recognition by its specific apoB/E receptor expressed on most cell types except macrophages. LDL modification alters their affinity for the apoB/E receptor and deviates their metabolism towards scavenger receptor-bearing cells (macrophages and smooth muscle cells), which are progressively transformed into foam cells (Steinbrecher, 1999). The accumulation of foam cells leads to the formation of fatty streaks that are characteristic of the early atherosclerotic lesions (Ross, 1993). Like 4-HNE and MDA, methylglyoxal and glyoxal may directly modify LDLs and deviate their metabolism towards macrophages (Brown *et al*., 2005).

Moreover, the degradation of oxLDLs in lysosomes is slowed down, possibly because 4-HNE is able to modify and inactivate the lysosomal cathepsin B (Hoppe *et al*., 1994).

Beside their metabolic deviation, oxLDLs exhibit a large variety of biological and atherogenic properties involved in the activation of inflammatory, mitogenic or proapoptotic pathways (Hajjar and Haberland, 1997; Uchida, 2000). The biological activity of oxLDLs depends on the presence of lipid peroxidation products such as oxysterols, lipoperoxides, lysophospholipids and reactive aldehydes (4-HNE, MDA, acrolein or methylglyoxal). These agents can mimic the toxic, inflammatory or mitogenic signalling mediated by oxLDLs (Leonarduzzi *et al*., 2005).

Modification of tyrosine kinase receptors and cell cycle

4-HNE added to cultured cells exhibits a clear dose-dependent effect, since physiological concentration of 4-HNE has growth-regulating effect, whereas higher concentration is primarily cytotoxic (Zarkovic *et al*., 1993).

4-HNE present in oxLDLs or exogenously added to the culture medium induces both modification and dysfunction of tyrosine kinase receptors (TKRs) such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) in a biphasic manner. 4-HNE, at physiological and moderate concentrations ($<1\,\mu$M and 1–10 μM, respectively) triggers a sustained activation of TKRs EGFR and PDGFR (Suc *et al*., 1997; Escargueil-Blanc *et al*., 2001). 4-HNE involved in TKRs modification originates from oxLDLs (as evidenced by the use of oxLDLs radiolabelled with [^{3}H] hydroxynonenal) and from the lipid peroxidation of cell membrane (Escargueil-Blanc *et al*., 2001). The mechanism of TKRs activation involves the formation of 4-HNE adducts on the receptor, which triggers TKR autophosphorylation and the activation of the downstream signalling pathway, extracellular signal-regulated kinase (ERK)1/2 phosphorylation and cell cycle progression (Suc *et al*., 1997; Escargueil-Blanc *et al*., 2001). Modification of purified TKRs by 4-HNE *in vitro* triggers the activation of the receptor, thereby demonstrating that TKR modification and activation are causally related (Suc *et al*., 1997; Escargueil-Blanc *et al*., 2001). From these observations it can be speculated that, at low (physiological ?) concentrations, 4-HNE can act as a growth

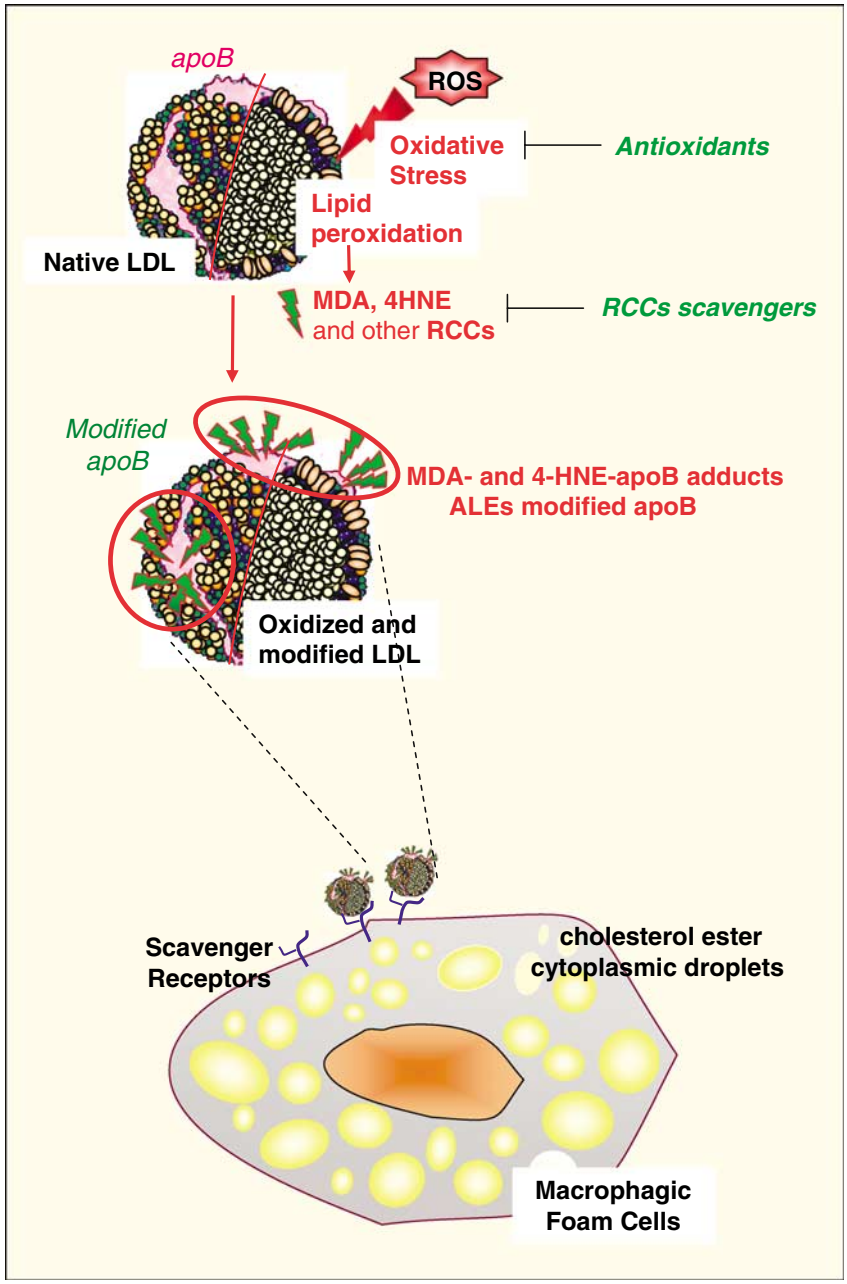

Figure 5 Modification of LDL and formation of foam cells. ROS induce the oxidation of PUFA in LDLs. Lipid peroxidation leads to the formation of aldehydic compounds such as 4-HNE and MDA. These RCCs are able to react with lysin residues of apoB leading to modified apoB. The affinity of modified LDL for the apoB/E receptor decreases when apoB modification increases. Modified oxidized LDL are deviated towards the scavenger receptor pathway of macrophages, which are loaded with cholesterol esters and are transformed into foam cells, the signature of the initial step of atherosclerosis (Brown and Goldstein, 1983; Steinberg *et al.*, 1989, 1997; Esterbauer *et al.*, 1990, 1991). LDL, low-density lipoprotein, ROS, reactive oxygen species; PUFAs, polyunsaturated fatty acids; 4-HNE, 4-hydroxynonenal; MDA, malondialdehyde; RCCs, reactive carbonyl compounds.

factor and promotes cell proliferation. However, high concentrations of 4-HNE inhibit cell proliferation mediated by growth factor receptors EGFR and PDGFR (Liu *et al.*, 1999; Vindis *et al.*, 2006), though a two-fold increase of the c-fos mRNA level was observed as a protective but abortive stress-induced response (Kreuzer *et al.*, 1998). This inhibitory effect of 4-HNE on growth factor-mediated cell proliferation is in agreement with the progressive desensitization of PDGFRβ to its own ligand PDGF-BB, observed in smooth muscle cell in prolonged contact with oxLDL or 4-HNE (at concentrations $>10\,\mu$M). The desensitization of PDGFRβ is characterized by an inhibition of the PDGF-induced PDGFRβ autophosphorylation, of the signalling cascade and of cell proliferation (Vindis *et al.*, 2006). Inhibition of cell cycle progression, reported in leukaemic cells, is mediated by a decrease in expression of cyclin D1, D2 and A and an increase in the expression of the cycline kinase inhibitor p21, thereby inducing an accumulation of cells in the G_0/G_1

phase of the cell cycle (Barrera *et al*., 2004). 4-HNE induces an accumulation of the hypophosphorylated form of the retinoblastoma tumour-suppressor gene product hyperphosphorylated retinoblastoma, which binds and inactivates the E2F transcription factors, represses the transcription and induces cell cycle arrest (Barrera *et al*., 2004). Moreover, 4-HNE inhibits the expression of the protooncogene c-myc in HL-60, without affecting the expression of c-fos (Barrera *et al*., 1996). Higher toxic concentrations of 4-HNE (50 μM) enhanced c-fos transcription while cell proliferation is inhibited (Kreuzer *et al*., 1997). 4-HNE and acrolein adducts on PDGFR occur also *in vivo* in atherosclerotic lesions from hypercholesterolemic rabbits, apoE−/− mice and human patients (Vindis *et al*., 2006), which suggests that 4-HNE may impair receptor tyrosine kinase (RTK) signalling (PDGFR) within the vascular wall, and may potentially disturb PDGFR-mediated responses (proliferation, cell migration and wound healing). These biological effects are not restricted to 4-HNE, since methylglyoxal is also able to inhibit various RTKs, including EGFR, PDGFR and insulin receptor, suggesting a role for this modification in accelerated atherosclerosis in diabetes (Portero-Otin *et al*., 2002; Riboulet-Chavey *et al*., 2006; Cantero *et al*., 2007).

Other signalling protein kinases

Sub-cytotoxic concentrations of 4-HNE are known to directly or indirectly alter a variety of cell signalling kinases (Leonarduzzi *et al*., 2004). Only few reports provide information on the molecular mechanisms and on the identity of proteins that are targeted by 4-HNE. Sub-toxic concentrations of 4-HNE induce the expression of antioxidant genes such as heme-oxygenase and thioredoxine-1 via the activation of the mitogen-activated protein kinase (MAPK) pathway and the transcription factor Nrf2 (Chen *et al*., 2005; Siow *et al*., 2007), suggesting that 4-HNE may promote adaptive response to oxidative stress. Low 4-HNE concentrations induce the release of monocyte chemotactic protein-1 by macrophages, through an activation of PKCβ (Nitti *et al*., 2002), whereas the activation of PKCδ and Jun N-terminal kinase is rather associated with apoptosis via an upregulation of the activator protein-1 DNA binding transcription factor (Castello *et al*., 2005). More generally, the activation of c-Jun N-terminal kinase (JNK) and p38 kinase pathways by 4-HNE is associated with the activation of transcription factors initiating cellular responses including cell proliferation, inflammatory responses, proteasomal-mediated protein degradation and apoptosis (Petersen and Doorn, 2004; Leonarduzzi *et al*., 2005).

Proteasome

Proteasome is activated by oxidative stress (Grune and Davies, 2003) and by low concentrations of oxLDLs (Robbesyn *et al*., 2003). In contrast, HNE-modified proteins are poorly degraded by proteasome and tend to inhibit the proteolytic activity (Grune and Davies, 2003). The extensive modification of cellular proteins by 4-HNE and by the related aldehydes leads to the formation of protein aggregates that accumulate in cells and are not degraded by the proteasome (Sitte *et al*., 2000; Grune and Davies, 2003). The decreased degradation of modified protein and their accumulation could result from a direct inhibition of proteasome by oxidized and cross-linked proteins (Sitte *et al*., 2000; Grune and Davies, 2003), and by 4-HNE-modified proteins (Friguet, 2006). Though the modification of proteasome is not observed at low or moderate 4-HNE concentrations (Grune and Davies, 2003), higher concentrations may directly form adducts on the three proteolytic activities of proteasome (trypsin, chymotrypsine and peptidylglutamyl peptide hydrolase) (Okada *et al*., 1999; Ferrington and Kapphahn, 2004), which readily inhibits the enzymatic activity of proteasome and contributes to the accumulation of modified proteins (Grune and Davies, 2003), and to apoptosis mediated by oxLDL (Vieira *et al*., 2000). For instance, the reduction in the chymotrypsin-like activity could be explained by the modification of Cys residues, as mimicked by treatment of purified proteasome sub-units with the sulfhydryl-reactive chemical N-ethylmaleimide (Kapphahn *et al*., 2007). The inhibition of proteasome contributes to the pathogenicity of diseases characterized by an accumulation of cross-linked proteins, such as Alzheimer's disease, in which HNE-modified amyloid β-peptide accumulates and efficiently inhibits the proteasome (Shringarpure *et al*., 2000). The accumulation of ubiquitinated modified proteins resulting from proteasome inhibition in neuronal cells triggers a proinflammatory response characterized by an upregulation of COX-2 and the production of prostaglandin PGE2, which contributes to neurodegeneration (Rockwell *et al*., 2000).

The ubiquitination conjugation process may be not necessary for the degradation of oxidized protein by proteasome, (Shringarpure *et al*., 2003), but is involved in the degradation of cellular proteins in contact with oxLDL or 4-HNE, since its inhibition potentiates apoptosis (Vieira *et al*., 2000). This ubiquitination process is not altered by 4-HNE (Vieira *et al*., 2000), and could occur at a faster rate than that of native proteins, as reported for 4-HNE-modified α,B-crystallin (Marques *et al*., 2004). In this paper, the authors describe another ubiquitin/lysosomal pathway that could degrade HNE-modified proteins independently of proteasome (Marques *et al*., 2004). However, other reports indicate that the lysosomal degradation of modified proteins by lysosomal cathepsin is impaired in aging and in pathological situations leading to the accumulation of modified proteins (Sitte *et al*., 2000).

NF-κB

The pro-inflammatory transcription factor NF-κB (nuclear factor-κB) is a direct regulator of proinflammatory and anti-inflammatory genes, cell survival and proliferation (de Winther *et al*., 2005). The activation of NF-κB requires the phosphorylation of its inhibitor κB (IκB), which is necessary for its degradation by the ubiquitin–proteasome pathway (Roff *et al*., 1996). 4-HNE and acrolein inhibit the activation of NF-κB, either via a direct inhibitory effect on proteasome or through the inhibition of an upstream step required for the phosphorylation of IκB as reported in human monocytic cells, in which 4-HNE inhibits the activation of NF-κB induced by lipopolysaccharide, interleukin-1β and phorbol

ester (Page *et al*., 1999). Conversely, 4-HNE prevents the activation of NF-κB elicited by Chlamydia pneumoniae by inhibiting the phosphorylation of IκB and its subsequent proteolysis (Donath *et al*., 2002). A potential mechanism has been proposed recently by Valacchi *et al*. (2005), who report that the inhibition of interferon-α (TNFα)-induced activation by acrolein could be due to the modification of IKKβ-subunit by acrolein. In contrast, aldehydes may induce inflammation via an activation of NF-κB, as shown for 4-HHE that activates NF-κB via the IκB kinase/NF-κB inducing kinase pathway. This mechanism involves an upstream activation of p38 MAPK and ERK1/2 kinase (Je *et al*., 2004).

Apoptosis signalling

Elevated concentrations of 4-HNE or acrolein ($>20\,\mu$M) are highly toxic for most cell types. The mechanism of apoptosis elicited by ALE precursors involves various effects, including signalling or protein modification. The activation of JNK has been particularly investigated in the antiproliferative and apoptotic effect of 4-HNE (Yang *et al*., 2003). This JNK pathway plays a major role in the cooperative apoptotic effect of tissue growth factor β-1 (TGFβ-1) and 4-HNE on colon cancer cell lines (Biasi *et al*., 2006). Both acrolein and 4-HNE increase the levels of the phosphorylated form of transcription factors c-jun (which promotes apoptosis) and CRE-binding protein (CREB) (involved in survival), but decrease the activity of the CREB-responsive promoters (while increasing c-jun responsive promoter), which contributes to neuron degeneration and apoptosis (Pugazhenthi *et al*., 2006). Methylglyoxal and glyoxal are pro-apoptotic through mechanisms involving calcium deregulation (Jan *et al*., 2005), GSH depletion, oxidative stress, and the activation of stress kinases p38 and JNK (Fukunaga *et al*., 2005; Chan *et al*., 2007).

4-HNE increases the mRNA and protein expression of the pro-apoptotic adaptors/regulators FasR, FasL, Bax, and caspases-1, -2, -3 and -8 (Choudhary *et al*., 2002; de Villiers *et al*., 2007). In human lens cultured cells (HLE B-3), 4-HNE adducts are correlated with the induction of Fas, the activation of JNK and caspase 3, while the transfection of the α-class GST mGSTA4a (which neutralizes 4-HNE) inhibits Fas expression (Cheng *et al*., 2001). The mechanism of cell death evoked by these aldehydes could also involve the generation of peroxynitrite ($ONOO^-$), as reported for 4-HHE (Lee *et al*., 2004) and for methylglyoxal (de Arriba *et al*., 2006).

4-HNE impairs the mitochondrial function, via the alteration of GSH metabolism and the induction of massive mitochondrial oxidative stress (Lee *et al*., 2006; Raza and John, 2006). More specifically, moderately elevated concentrations of 4-HNE or very low doses of 4-HHE trigger a calcium-mediated induction of the mitochondrial transition pore (Kristal *et al*., 1996). In addition, *in vitro* experiments on isolated mitochondria or reconstituted models for the adenine nucleotide translocator (ANT) pretreated with 4-HNE or 4-HHE indicate that the modification of ANT by these aldehydes impairs its function and activity (Chen *et al*., 1995). Lastly, 4-HNE alters mitochondrial calcium uptake and cytosolic calcium homoeostasis, which results in necrosis or apoptosis. This mechanism is involved in neuronal cell death (Kruman and Mattson, 1999).

ALEs and ALE precursors in ageing and diseases

Considerable evidence suggests the involvement for ALEs in ageing and age-related diseases. Ageing process is associated with an imbalance between increased oxidative stress and progressive decrease in antioxidant defences, as well as the accumulation of modified proteins, due to increased protein damage and/or decreased degradation by proteasome (Friguet, 2006). More than simple markers of lipid peroxidation, ALEs may participate in initiation and progression of diseases, including cardiovascular diseases, diabetes, cancer, inflammatory and neurodegenerative disorders.

ALEs in cardiovascular and related diseases

The oxidative theory of atherosclerosis proposed by Steinberg (1997) 25 years ago postulates that 4-HNE- and MDA-modified LDLs are directly involved in the mechanisms of fatty streak formation, an early step of atherogenesis. The detection of 4-HNE and MDA adducts, and oxLDLs within the plaque is an hallmark in atherosclerosis (Palinski *et al*., 1989; Torzewski *et al*., 1998). The presence of auto-antibodies recognizing MDA-modified LDLs has been reported in the plasma, but their role on the development of atherosclerosis is debated (Fredrikson *et al*., 2006). Nonetheless, the detection in human plasma and atherosclerotic lesions of auto-antibodies directed against 4-HNE- or MDA-modified LDLs is considered as a marker of evolution for vascular atherosclerotic process (Tsimikas, 2006). Circulating modified LDLs constitute new and suitable prognostic indicators of cardiovascular diseases, for example acute coronary syndromes, vulnerable plaques, pre-clinical or accelerated atherosclerosis and chronic renal failure in diabetes (Makita *et al*., 1996; Fraley and Tsimikas, 2006). Chronic renal failure complications including renal toxicity, ischaemia/reperfusion and myocardial injury result in a massive oxidative stress and the generation of MDA and 4-HNE, which deplete antioxidants defences, modify enzyme function and mitochondrial respiration by forming adducts on proteins (Siems *et al*., 2002). The presence of MDA and 4-HNE adducts in dialysed patients is considered as an additional risk factor for cardiovascular complications (Carluccio *et al*., 2002).

Coronary heart diseases and stroke due to accelerated atherosclerosis are the principal cause of mortality in diabetes. LDL modification by AGE and ALE precursors is detected in the plasma of diabetic patients (Makita *et al*., 1996) and is thought to play a role in the development and progression of accelerated atherosclerosis in diabetes. Moreover, diabetic cataract is associated with increased deposition of AGEs/ALEs-modified proteins in lens. The deposition of 4-HNE and MDA adducts in the lens of aged rats or submitted to high oxidative stress is directly correlated to the apoptosis of lens cells and cataract formation (Marsili *et al*., 2004). At last, 4-HNE could contribute to the mechanisms of obesity and insulin resistance, via the modification of adipose

regulatory proteins (for example, adipocyte fatty acid-binding protein) (Grimsrud *et al*., 2007).

Neurodegenerative diseases

Increasing evidences suggest that oxidative stress is involved in the pathogenesis of neurodegenerative diseases, including Alzheimer (Lovell and Markesbery, 2006), Parkinson (Jenner, 2003), Creutzfeld–Jacob and prion diseases (Brown, 2005). It is likely that the mechanisms involved in neurodegeneration are multiple, and it is not clear so far if oxidative stress is the cause or consequence of the neuronal death, as it may occur during apoptosis and in the early steps or in advanced stages of the disease. Moreover, the detection of aldehyde adducts in the brain of neurodegenerative diseases strongly suggests the occurrence of an oxidative stress during brain degeneration (Zarkovic, 2003).

In Alzheimer's disease, amyloid β-peptide induces an oxidative stress, which results in the formation of 4-HNE and acrolein adducts, and the accumulation of modified proteins (Sayre *et al*., 1997; Smith *et al*., 1998). 4-HNE forms adducts on Lys residues of proteins of axonal neurofilaments (Wataya *et al*., 2002) and induces a dysfunction of membrane calcium-ATPases, and of glucose and glutamate transporters, which increases calcium influx, disrupts the synaptic calcium homoeostasis and finally leads to apoptosis (Mattson and Chan, 2003).

In Parkinson's disease, the oxidative stress contributes to mitochondrial dysfunction and degeneration of dopaminergic cells (Jenner, 2003). 4-HNE is formed during the lipid peroxidation process and reacts with proteins of the nigral neurons (Yoritaka *et al*., 1996). In addition, 4-HNE- and peroxynitrite-induced modification of proteins associated with the defect of the ubiquitin–proteasome system leads to the accumulation of modified proteins and contributes to neuronal cell death (Jenner, 2003).

The neurodegenerative process in prion diseases may result, at least in part, from a defect in antioxidant function of the mutant form of prion protein (Brown, 2005). 4-HNE is generated in brains of human patients affected with Creutzfeldt–Jakob disease, and in scrapie-infected mice. 4-HNE adducts are detected in astrocytes, not in neurons, and their role in the progression of the prion disease is not elucidated so far (Andreoletti *et al*., 2002).

Cancer

The role of lipid peroxidation in cancer is debated because, in the majority of tumours, the lipid composition of membrane is modified with decreased PUFA level and increased cholesterol content. Moreover, an increase in antioxidants and antioxidant defences is often observed, thus indicating that lipid peroxidation could be reduced in these diseases (Dianzani, 1989). In addition, *in vivo* studies on human colon adenocarcinoma show a reduced expression of TGFβ-1 correlated with a decrease in 4-HNE–protein adducts. This could mean that neoplastic progression is better under these conditions, since TGFβ-1 and 4-HNE cooperate for inducing the apoptosis of colon cell via activation of the JNK pathway (Biasi *et al*., 2006).

On the other hand, 4-HNE itself is mutagenic (it can form adducts on guanine) and carcinogenic for hepatocytes (Weber *et al*., 2003). Low 4-HNE concentrations trigger proliferative and inflammatory responses, which could play a role in tumour growth, this being supported by the detection of 4-HNE adducts in various tumour cells (Dianzani, 1989). Moreover, high heam-iron intake (reproduced by high meat intake) is associated with lipid peroxidation and 4-HNE generation, potentially involved in the promotion of colon cancer carcinogenesis (Pierre *et al*., 2007). The same authors propose the detection of an urinary metabolite of 4-HNE, the dihydroxynonane mercapturic acid, as a new biomarker suitable for the determination of pre-neoplasic lesions (Pierre *et al*., 2006).

Chronic inflammatory diseases

MDA and 4-HNE adducts are detected in osteoarthritic synovial cartilage (Shah *et al*., 2005), and contribute to cartilage degradation, by modifying type II collagen, which increases its degradation by metalloprotease MMP13 (Morquette *et al*., 2006). Moreover, 4-HNE alters osteoarthritic osteoblast activity by triggering diverse cellular responses such as increased osteocalcin and type I collagen synthesis, inhibition of alkaline phosphatase activity, induction of COX-2 expression and prostaglandin E2 release (Shi *et al*., 2006). Protein oxidation is involved in the pathogenesis of systemic lupus erythematosus (Morgan *et al*., 2005), and the detection of 4-HNE-modified proteins in the plasma of the affected patients has been proposed as a marker for the evolution of this disease (Grune *et al*., 1997).

Oxidative stress and lipid peroxidation contribute to the pathogenesis of asthma, acute lung inflammation and allergic airway inflammation (Boldogh *et al*., 2005; Castro *et al*., 2006). In these diseases, the exact role of 4-HNE is not yet established and its targets are not identified, but the overexpression of heme-oxygenase reduces oxidative stress, 4-HNE adducts formation and the progression of inflammation (Almolki *et al*., 2004). Increased MDA and 4-HNE protein adducts, correlated with decreased levels of GSH and antioxidants vitamins E and C, is a constant observation in chronic viral and alcoholic hepatitis (Loguercio and Federico, 2003). Conversely, the reduced expression of GST in bile duct leads to the accumulation of 4-HNE, and contributes to the pathogenic process of primary biliary cirrhosis (Tsuneyama *et al*., 2002).

Pharmacological inhibitors of ALE formation

The pharmacological inhibition of ALE formation involves several approaches including the use of transition metal chelators and antioxidants, which block oxidative stress, lipid peroxidation, and the generation of aldehydes, and the use of carbonyl scavengers, which interact more specifically with aldehydes and neutralize the formation of adducts. Most studies have been focussed on AGE inhibitors, as recently reviewed by Peyroux and Sternberg (2006), whereas ALE inhibitors have been less investigated so far. Although most inhibitors efficient on AGE formation may potentially

inhibit ALE formation (Baynes and Thorpe, 2000), this review is focussed on the main pharmacological agents, which have been reported to neutralize ALE precursors generated from lipid peroxidation.

Hydrazine derivatives

Hydrazine. It is widely used in chemical synthesis as it condensates rapidly with the carbonyl group of ketone or aldehyde to form a methylene or methyl group via a hydrazone intermediate.

DNPH is a hydrazine derivative largely utilized for the quantitative detection of carbonyl groups (particularly 4-HNE) in tissues and biological fluids, using HPLC and spectrophotometric methods or for immunohistochemistry and western blotting using DNPH-labelled antibodies (Esterbauer *et al*., 1991; Waeg *et al*., 1996). DNPH reacts with aldehydes at acidic pH, which facilitates the rupture of the covalent bond aldehyde protein, and the binding of aldehyde on hydrazine, forming dinitrophenylhydrazones (Esterbauer *et al*., 1991). DNPH prevents *in vitro* the formation and the accumulation of acrolein and 4-HNE adducts on cellular proteins from cultured vascular cells, as well as the cytotoxicity of oxLDLs (Escargueil-Blanc *et al*., 2001). Anyway, the use of DNPH for *in vivo* trials in animals is limited because of its high mutagenic and toxic properties (Brooke *et al*., 1997).

Hydralazine (1-hydrazinophthalazine monohydrochloride). It is a hydrazine derivative used as antihypertensive, and regarded for years as a drug of choice for the treatment of severe hypertension in pregnancy (Montan, 2004). Hydralazine and dihydralazine are efficient in trapping aldehyde-adducted proteins. It is particularly efficient in trapping acrolein, resulting in a strong protection against acrolein-induced hepatotoxicity (Burcham *et al*., 2000, 2002; Kaminskas *et al*., 2004). Hydralazine traps 4-HNE adducts formed in smooth muscle cells in the presence of oxLDL, particularly on PDGF receptor that reverses the inhibition of PDGF signalling (Vindis *et al*., 2006). Hydralazine reverses *in vivo* the formation of 4-HNE and acrolein adducts on tissue proteins, particularly the modification of PDGFR in atherosclerotic aortas of hypercholesterolemic rabbits and of apoE−/− mice. This protective effect could contribute to slow down the atherosclerotic process (Vindis *et al*., 2006). Hydralazine inhibits the modification of LDL induced by glyoxal and methylglyoxal, and prevents the formation and accumulation of foam cells (Brown *et al*., 2006), as well as renal damage in type II diabetes animal models (Nangaku *et al*., 2003). Hydralazine is a powerful antioxidant that inhibits the activation of NADH oxidase at the plasma membrane and the subsequent generation of ROS (Munzel *et al*., 1996).

Aminoguanidine (hydrazinecarboximidamide). It is an early-stage inhibitor of AGE generation, and one of the most efficient drugs able to prevent proteins cross-linking induced by AGE precursors. Aminoguanidine reacts *in vitro* and *in vivo* with the α-oxoaldehydes methylglyoxal and glyoxal and ALE precursors (MDA) to form 3-amino-1,2,4-triazine derivatives (Thornalley *et al*., 2000; Thornalley, 2003). It is a highly nucleophilic agent that reacts rapidly with glucose and pyruvate, but also with pyridoxal phosphate, thereby decreasing the availability of vitamin B6 (Chen *et al*., 2004). Pyridoxal-aminoguanidine, a Schiff base formed between pyridoxal and aminoguanidine, prevents the decrease in pyridoxal phosphate, without altering and rather enhancing the carbonyl scavenger activities of aminoguanidine (Chen *et al*., 2004).

Most beneficial effects of aminoguanidine are related to its carbonyl scavenger properties, which protect *in vitro* and *in vivo* against the deleterious effects of AGE and ALE precursors (Peyroux and Sternberg, 2006). Aminoguanidine has been proved efficient in experimental animal models for diabetes, in inhibiting pathological complications, such as nephropathies (prevention of albuminuria and glomerulonephritis), accelerated atherosclerosis (inhibition of lipid peroxidation), cataract (inhibition of AGE deposition in lens) and neurovascular complications (Peyroux and Sternberg, 2006).

In addition, the protective effect of aminoguanidine is largely due to its antioxidant and chelating properties (Price *et al*., 2001). For instance, aminoguanidine reduces the expression of growth factors TGFβ-1 and PDGF-β and of proinflammatory cytokines (TNFα) (Peyroux and Sternberg, 2006), probably by preventing oxidative stress and NF-κB activation. Aminoguanidine inhibits the activity of the inducible nitric oxide synthase, which is upregulated in diabetes and generates high NO levels leading to peroxynitrite generation and endothelial dysfunction (Bardell and MacLeod, 2001). Conversely, aminoguanidine inhibits the semicarbazide-sensitive oxidase, which contributes to the generation of methylglyoxal and formaldehyde in diabetes (Yu and Zuo, 1997). Like hydralazine, aminoguanidine blocks the modification of LDL induced by methylglyoxal and MDA, thereby preventing their metabolic deviation towards macrophages (Brown *et al*., 2006). This protective effect (inhibition of apoB carbonylation) could result from its antioxidant properties, in addition to the carbonyl-scavenger activity (Jedidi *et al*., 2003).

Because aminoguanidine exhibits extensive beneficial effect in pre-clinical studies in experimental animal models for diabetes, several clinical trials in humans have been designed for evaluating its efficiency to slow down the progression of diabetes complications. These studies were not found conclusive, in part because of side-effects, and of weak carbonyl scavenger effects in human vascular tissues (Bolton *et al*., 2004).

OPB-9195 ((+/−)-2-isopropylidenehydrazono-4-oxo-thiazolidin-5-yla cetanilide). It is a hydrazine derivative that inhibits the formation of AGEs (pentosidine and CML) in the plasma of uremic and diabetic patients (Miyata *et al*., 2000). OPB-9195 is more efficient than aminoguanidine for trapping 4-HNE and MDA from arachidonate oxidation (Miyata *et al*., 2000). As for aminoguanidine, clinical trials were not conclusive and were stopped because of the side-effects of this compound, particularly pyridoxal depletion which resulted in vitamin B6 deficiency (Peyroux and Sternberg, 2006).

Vitamin B6 derivatives

Vitamin B6. It exhibits antioxidant properties as it participates in the maintenance of reduced GSH, which is a major antioxidant and a natural ALE precursor scavenger. Vitamin B6 acts as a cofactor in the synthesis of Cys (the rate-limiting precursor for GSH biosynthesis) (Grimble, 1997). Deficiency or low levels in vitamin B6 result in an increased homocysteine level and oxidative stress potentially involved in accelerated atherosclerosis in vitamin B6-deficient rats (Endo *et al*., 2006).

Pyridoxamine. It is one of the three natural forms of vitamin B6 (with pyridoxal and pyridoxine). Pyridoxamine prevents the formation of AGEs such as CML and CEL, and is a potent inhibitor of Lys modification by 4-HNE and MDA generated during LDL oxidation by copper (Onorato *et al*., 2000). It is a potent MDA scavenger, which also blocks the accumulation of the fluorescent MDA-related pigment, lipofuscin (Kang *et al*., 2006). Pyridoxamine inhibits NO-mediated apoptosis of insulin secreting RINm5F cells, which occurs via the formation of ALE precursors (for example, MDA) (Cahuana *et al*., 2003). Moreover, pyridoxamine has a lipid-lowering effect and protects against the development of nephropathy, neuropathy and vaculopathy in streptozotocin-induced diabetic rats. Like aminoguanidine, pyridoxamine represents a potentially useful agent for the treatment of diseases involving hyperlipemia and oxidative stress (Voziyan and Hudson, 2005).

Pyridoxal isonicotinoyl hydrazones. These classes of agents exhibit potent chelating properties against iron and block iron-induced oxidative stress and lipid peroxidation, in particular MDA formation. Their use is reserved to iron overload diseases and neurodegenerative disorders (Whitnall and Richardson, 2006).

Amino-acid derivatives

Carnosine (β-alanyl-L-histidine). It is a dipeptide of β-alanine and His, exhibiting antioxidant and carbonyl scavenger properties. Carnosine blocks the formation of MDA and methylglyoxal adducts in *in vitro* experimental models for lipid peroxidation and MDA-induced cytotoxicity in cultured brain endothelial cells (Hipkiss *et al*., 1997). At the cellular level, carnosine protected cultured human fibroblasts and rat brain endothelial cells against the toxic effects of MDA and AGEs. Interestingly, carnosine protects against amyloid peptide toxicity and DNA protein cross-linking in neurodegenerative disorders such as Alzheimer's disease, cardiovascular ischaemic damages and inflammatory diseases (Guiotto *et al*., 2005).

Histidyl hydrazide. It is a His analogue that selectively scavenges 4-HNE, and reduces the 4-HNE-induced apoptosis of cultured neurons, chemical hypoxia and glucose deprivation. This drug could be of interest in the treatment of related neurodegenerative diseases and 4-HNE-associated pathologies (Tang *et al*., 2007).

N-*acetyl cysteine*. It exhibits highly protective scavenging properties against 4-HNE and MDA. *N*-acetyl cysteine (NAC) pretreatment protects against MDA increase and GSH decrease in an animal model for Alzheimer (Fu *et al*., 2006), and prevents accelerated atherosclerosis in uremic apoE−/− mice (Ivanovski *et al*., 2005), as well as neuronal injury (Arakawa *et al*., 2006).

S-Adenosylmethionine. It is more potent than NAC for inhibiting 4-HNE adduct formation, and prevents efficiently hepatic toxicity induced by acetaminophen, which involves oxidative stress and lipid peroxidation (Valentovic *et al*., 2004).

Other therapeutic agents exhibiting protection against ALE formation

Angiotensin converting enzyme inhibitors. Angiotensin converting enzyme inhibitors exhibit antioxidant properties and block LDL oxidation, lipid peroxidation and the generation of MDA and 4-HNE (Hayek *et al*., 1999). ACE inhibitors (Captopril, Enalapril and Fosinopril) exhibit a potent antiatherogenic effect in apoE−/− mice, due to not only blood pressure reduction but also to their protective effect against LDL oxidation (Hayek *et al*., 1998, 1999). Moreover, a direct MDA-scavenging effect has been reported for Captopril (Altuntas *et al*., 1995).

AT1 angiotensin receptor inhibitors (Losartan, Candesartan). These inhibit the formation of circulating MDA-modified LDLs in human diabetic patients and the modification by MDA of lungs in rats affected with bleomycin-induced pulmonary fibrosis (Hayek *et al*., 1999; Yao *et al*., 2006).

Antioxidants. These agents are efficient in preventing lipid peroxidation, thus the formation of ALEs on proteins. Among natural antioxidants involved in the detoxification of ALE precursors, GSH (Glu–Cys–Gly) exhibits a high reactivity for 4-HNE and is essential for maintaining antioxidants in an active state (α-tocopherol), and for protecting the cell against oxidative stress mediated by ROS (Petersen and Doorn, 2004). Though GSH can react spontaneously with 4-HNE via Michael addition, the reaction is much more rapid via the conjugation process catalysed by GST (EC 2.5.1.18), the highest activity being exhibited by GST A4-4 and GST 5.8 (Hubatsch *et al*., 1998; Petersen and Doorn, 2004). The conjugation catalysed by GSTs transforms 4-HNE into a GSH conjugate, thus decreases GSH intracellular concentration, while GSTs may also limit 4-HNE generation by reducing hydroperoxide formed during lipid peroxidation (Awasthi *et al*., 2004). GST4 activity is reduced in obese mice, which is correlated with the modification by 4-HNE of a fatty acid-binding protein on Cys, thus supporting the existence of links between oxidative stress and insulin resistance (Grimsrud *et al*., 2007). Interestingly, low doses of 4-HNE can induce the expression of the glutamate–Cys ligase (a rate-limiting enzyme in GSH biosynthesis), through a mechanism implying the EpRE–Nrf2 signalling pathway (Zhang *et al*., 2007).

Pretreatment of neurons with high NAC concentrations prevents 4-HNE-induced neuronal death, and suppresses the

decrease in intracellular levels of GSH and sulfhydryl groups induced by 4-HNE. Lower NAC concentrations potentiated the protective effect of the seleno-organic GSH peroxidase mimetic Ebselen (Arakawa *et al*., 2006), though Ebselen itself prevents the generation of 4-HNE adducts formed during cerebral ischaemia (Imai *et al*., 2003), or alcohol-induced liver injury (Kono *et al*., 2001). These data are interesting as they propose a dual protective mechanism for these drugs via (i) their carbonyl scavenger effect against ALE precursors (4-HNE) and (ii) the maintenance of an efficient level in natural intracellular antioxidants (GSH), and the natural protection of the cells against oxidative stress.

However, antioxidants cannot neutralize the effects of ALE precursors once adducts are formed. For instance, trolox and phenolic acids cannot protect against the modification of TKRs induced by 4-HNE (Vacaresse *et al*., 2001). *In vivo* studies indicate that the protective effect of antioxidants on the formation of 4-HNE, MDA or acrolein in atherosclerotic plaques or in neurodegenerative diseases is variable, while agents efficient *in vitro* or in pre-clinical studies fail to protect significantly once tested in human patients (Peyroux and Sternberg, 2006). U-101033E (2,4-diaminopyrrolopyrimidine) is highly efficient in inhibiting 4-HNE or MDA generation (Rohn *et al*., 1998), but its inhibitory effect on ALE formation has not been studied *in vivo*. Vitamin E failed to protect humans from cardiovascular disease outcome and its antiatherogenic effect in apoE−/− is controversial (Suarna *et al*., 2006). Pyrrolidine dithiocarbamate blocks efficiently lipid peroxidation (MDA) in chronic inflammation (collagen-induced arthritis) (Cuzzocrea *et al*., 2002) and cerulein-induced pancreatitis (Virlos *et al*., 2003). Polyphenols reduce hyperlipemia and inhibit lipid peroxidation and atherosclerosis development in diabetic LDL receptor KO mice (Zang *et al*., 2006). Resveratrol, a red wine polyphenol, exhibits protective properties against lipid peroxidation and ALE formation in experimental models for numerous diseases including atherosclerosis, diabetes and Alzheimer diseases (Anekonda, 2006). However, most agents were not tested in human patients, and any correlation between their protective effect on ALE generation and the progression of the disease remains speculative.

Conclusion

ALEs are formed in a large variety of diseases and represent a suitable marker of lipid peroxidation. Their deleterious effect on proteins and their own signalling properties suggest that ALEs also contribute to initiate several diseases or aggravate their severity. Obviously, the early inhibition of lipid peroxidation and ALE formation by antioxidants blocks efficiently atherogenesis in animal models, whereas antioxidants fail to protect on more advanced states and in therapeutic human trials. Studies using AGE/ALE-precursor scavengers on the progression of diabetes complications were not conclusive in humans, and led to variable conclusions in animals. The effect of drugs like hydralazine on cardiovascular, inflammatory or neurodegenerative diseases has not been investigated in humans, thus it is not known if ALE-precursor scavengers could be beneficial or not on the late steps of ALEs-associated diseases. The synthesis and development of new drugs sharing both potent antioxidant and carbonyl scavenger properties should allow (i) to better understand the implication of ALEs in the advanced steps of diseases and (ii) to better evaluate the potential therapeutic interest of this class of carbonyl scavengers. The new therapeutical perspectives of these new drugs will also depend on their influence on natural cellular antioxidants and their ability to protect or regenerate their protective effect against oxidative stress.

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Conflict of interest

The authors state no conflict of interest.

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