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REVIEW

Role of 4-Hydroxy-*trans*-2-nonenal in Cell Functions

E. E. Dubinina[1]* and V. A. Dadali[2]

[1]*Bekhterev St. Petersburg Psychoneurological Research Institute, ul. Bekhtereva 3, 192019 St. Petersburg, Russia; fax: (812) 412-7127; E-mail: eedubinina@rambler.ru*
[2]*Mechnikov St. Petersburg State Medical Academy, Piskarevsky pr. 47, 195067 St. Petersburg, Russia; fax: (812) 543-0208; E-mail: yudadali@yandex.ru*

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Abstract—The role of lipid peroxidation product 4-hydroxy-*trans*-2-nonenal (4-HNE) in functional activity of cells under normal and different pathological conditions is discussed. Different pathways of 4-HNE metabolism in tissues are analyzed, with particular focus on the role the glutathione system in this process. 4-HNE is implicated in regulation of cell growth, proliferation, differentiation, and apoptosis. 4-HNE and metabolic products of other antioxidants (carotenoids) resemble each other in chemical nature of the product and influence general pathways of signal transduction. Manifestation of 4-HNE toxicity under oxidative stress conditions is regarded as a link to many diseases whose pathogenesis is connected with modifications of proteins and nucleic acids.

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Under normal conditions the functional activity of cells is associated with ceaseless free-radical processes coupled with generation of reactive oxygen species (ROS) and their metabolites, such as lipid peroxidation (LPO) products. ROS are now known to serve as signaling molecules in most plant and animal cells and tissues. Many metabolic processes determining the functional state of a cell are supported by activation of multicatalytic complexes in which regulation ROS play an important role [1-5].

ROS exert a multitude of effects on the functional activity of cells. They are implicated in regulation of apoptosis [6-8], cell adhesion [9], and blood clotting [10], induce or suppress expression of many genes [11-13], regulate cell growth and differentiation [14, 15], etc.

Quite recently data have appeared on the effects of final LPO products, such as malonic dialdehyde (MDA), 4-hydroxy-*trans*-2-nonenal (4-HNE), and other alkenals, on vital functions of cells. In particular, 4-HNE, the major water-soluble LPO product, acts as a signaling molecule under physiological conditions by influencing functional activity of cells via signal transduction and gene expression.

CHARACTERISTICS OF 4-HYDROXY-*trans*-2-NONENAL METABOLISM

Lipid peroxidation can occur through pathways of free radical-mediated, free radical-independent nonenzymatic and enzymatic oxidation accompanied by formation of various intermediate, secondary, and final products [16]. The latter comprise a large group of reactive low molecular weight aldehyde members of three families: 2-alkenals, 4-hydroxy-2-alkenals, and ketoaldehydes. 2-Alkenals, such as acrolein, crotonaldehyde, and hexenal, are the most reactive. 4-Hydroxy-2-alkenals include 4-HNE and 4-hydroxy-2-hexenal (HHE). Ketoaldehydes include MDA, glyoxal, and 4-oxo-2-nonenal (ONE) (Fig. 1) [17].

4-HNE is a major peroxidation product of ω-6 polyunsaturated fatty acids such as linoleic and arachi-

Abbreviations: AD, Alzheimer's disease; ALDH, NAD^+-dependent aldehyde dehydrogenase; AO, antioxidant; GCL, glutamate-cysteine ligase (γ-glutamyl-L-cysteine synthetase); GGTP, γ-glutamyl transpeptidase; GS, glutathione synthetase; 4-HNE, 4-hydroxy-*trans*-2-nonenal; IL, interleukin; LPO, lipid peroxidation; MDA, malonic dialdehyde; Nrf2, zinc-binding protein, an NF-E2 related factor 2; OS, oxidative stress; PI3K, phosphatidylinositol 3-kinase; pRb, retinoblastoma protein; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; Shc, SHC-adaptor protein; TNF-α, tumor necrosis factor alpha.

* To whom correspondence should be addressed.

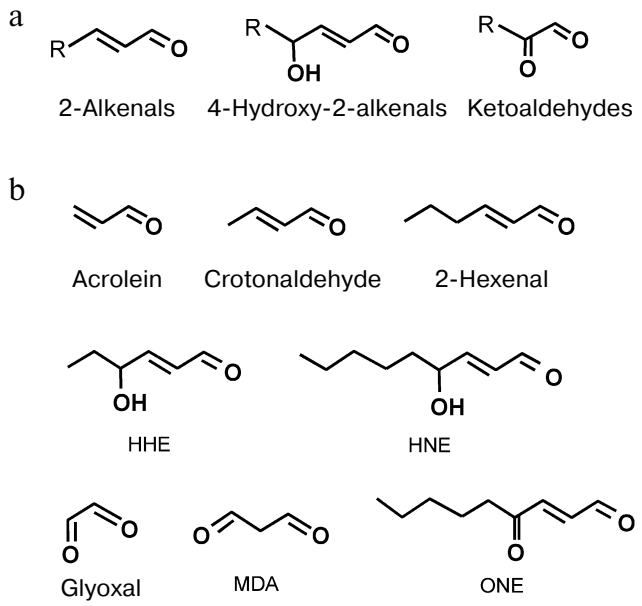


Fig. 1. Structures of reactive aldehydes (a) and aldehyde products of lipid peroxidation (b) [17].

donic acids (Scheme 1) [18-20]. The biological effect of 4-HNE depends on its chemical structure, lipophilicity, and donor–acceptor capability and does not depend on intensity of its metabolism in the cell. 4-HNE is more lipophilic than hydrophilic substances, which predisposes it to localization in the cell membrane.

Reactions influenced by conjugated double bonds in the 4-HNE molecule include Michael addition, reduction, and epoxidation. Michael addition involves not only thiol groups of cysteine or glutathione, but also amine groups of lysine, ethanolamine, and guanine, as well as the imidazole group of histidine. The epoxidation reaction proceeds in the presence of hydroperoxides, and its mechanism is not completely elucidated.

The carbonyl group of 4-HNE is associated with oxidation, reduction, formation of acetals and thioacetals with alcohols and thiols, respectively, and Schiff base formation as well. Schiff bases are formed due to the interaction of the 4-HNE with an amine group, for instance the amine group of lysine. They play an important role in cross-linking of oxidized proteins. Reactions coupled with the 4-HNE hydroxyl group yield cyclic hemiacetals or ketones and are secondary [21].

Both oxidation and reduction of the 4-HNE carbonyl group commonly occur enzymatically. In particular, 4-HNE oxidation by mitochondrial NAD^+-dependent aldehyde dehydrogenase (ALDH; EC 1.2.1.3) results in formation of 4-hydroxynon-2-enoic acid (4-HNA). 4-HNE as an ALDH substrate can act as its inhibitor. The Cys302 residue of the enzyme catalytic center can undergo modification due to the Michael reaction with 4-HNE. It is likely that inhibition of the enzyme is because of its decelerated turnover when 4-HNE is used as a substrate [22, 23].

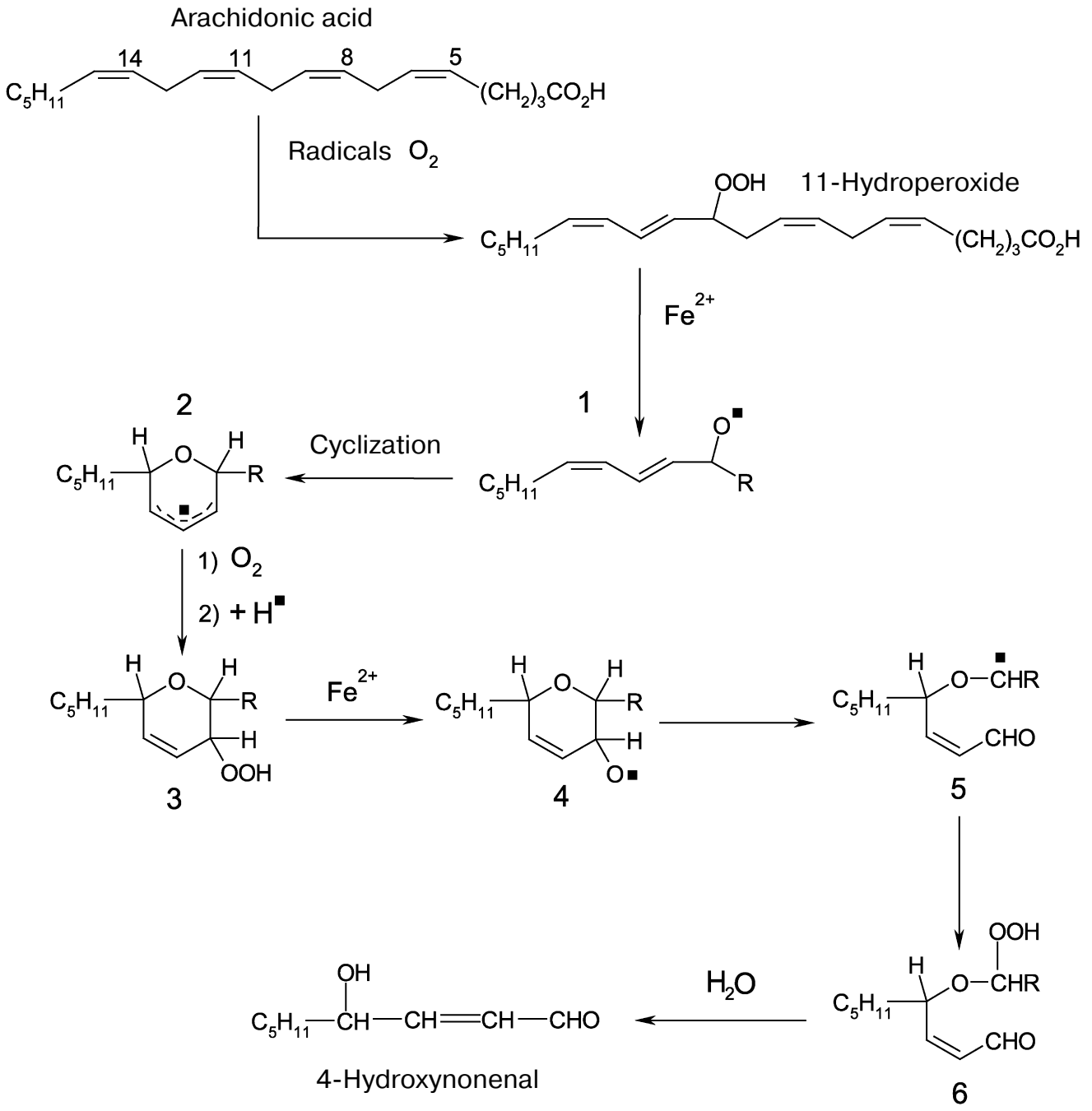
4-HNE is enzymatically reduced by various enzymes, such as members of the aldo/keto reductase family and alcohol dehydrogenases (EC 1.1.1.1). Aldehyde reductase (EC 1.1.1.2) and its subfamily members and aldose reductase (EC 1.1.1.21) catalyze the NADPH-dependent reduction of 4-HNE with formation of inactive 1,4-dihydroxy-2-nonene (DHN) [24-26].

Conjugation with reduced glutathione (GSH) plays an important role in 4-HNE metabolism. Although GSH can spontaneously interact with 4-HNE by Michael addition, the conjugation can occur due to the enzymatic reaction catalyzed by glutathione S-transferase (GST; EC 2.5.1.18) possessing high affinity to the aldehyde (Scheme 2) [27, 28]. Interestingly, GS-HNE is also a substrate of aldose reductase, which catalyzes NADPH-dependent reduction of the GS-HNE conjugate to produce glutathione-DHN (GS-DHN) [29, 30]. A recent discovery is the reduction of C=C double bond of 4-HNE by alkenal oxidoreductase (EC 1.3.1.48). This enzyme was found to have exceptionally high activity toward 4-HNE in the presence of NADPH. The reaction product is 4-hydroxynonanal (4-HAA), which rearranges to a lactone form [22].

All these data were obtained from *in vitro* experiments. Metabolism of 4-HNE can significantly differ in different cell types. In particular, in rat hepatocytes up to 50-60% 4-HNE is eliminated via conjugation with GSH, whereas only ~10% of 4-HNE undergoes oxidation/reduction. Other products of 4-HNE biotransformation (~30-40%) are not yet identified [30]. Another study on 4-HNE metabolism in hepatocytes suggests that 30% of 4-HNE is involved in formation of GSH-conjugates, 30% of 4-HNE is oxidized, 10% is reduced yielding DHN, and only 3% of 4-HNE is bound with proteins. Metabolism of 4-HNA is coupled with β-oxidation (7%) [31]. Subsequent study has shown that GS-DHN is the major product that is involved in ω-oxidation in liver [32].

In Kupffer cells, the metabolism of 4-HNE differs from that in hepatocytes. The metabolic rate is 100-fold lower. Formation of conjugates with glutathione is also decreased in Kupffer cells, possibly because of low content of GSH [33, 34].

In erythrocytes the major metabolic pathway of 4-HNE (70%) is conjugation with reduced glutathione, mainly catalyzed by GST. Subsequently, the conjugates can be reduced to form GS-DHN. This process is more intensive than spontaneous interaction between GSH and DHN. Reduction of both 4-HNE and its conjugates with glutathione is catalyzed by aldose reductase. It is considered that aldose reductase-mediated catalysis is an additional defense against the toxic effect of 4-HNE formed due to spontaneous dissociation of GS-HNE. Unlike hepatocytes, in which the contribution of 4-HNE reduction catalyzed by alcohol dehydrogenase is 20-30% of the

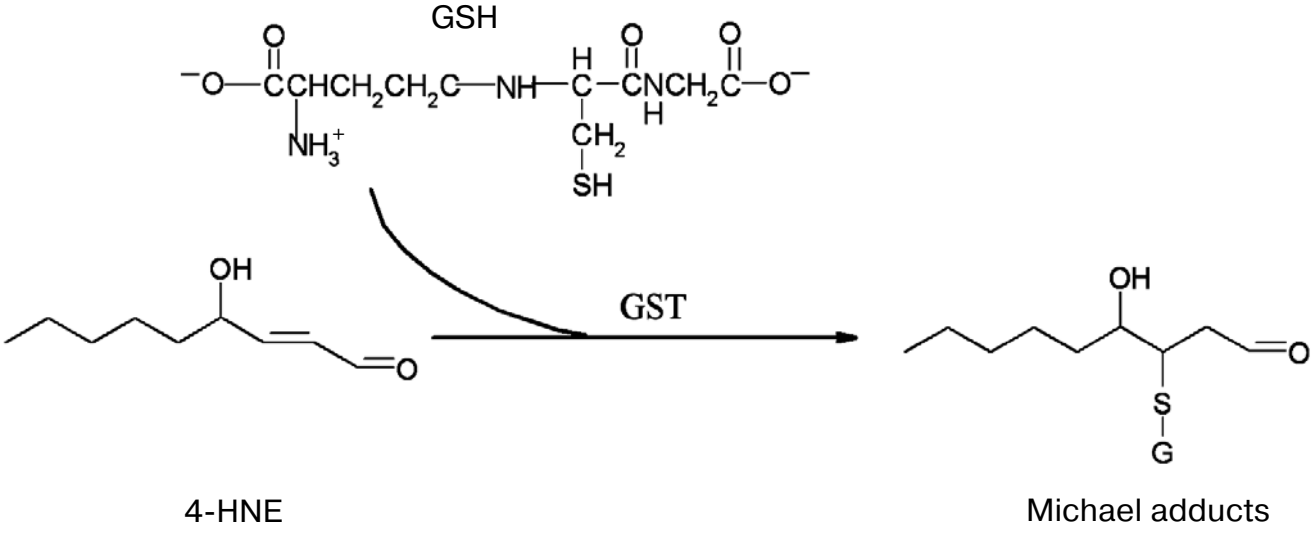


Presumed mechanism of 4-HNE formation during oxidation of arachidonic acid [19]

Scheme 1

overall 4-HNE metabolism, this process is not dominant in erythrocytes. A significant metabolic part (25%) of 4-HNE is formation of corresponding HNA-carbonic acid [35].

Analysis of data from the literature suggests high metabolic rate of 4-HNE in mammalian tissues. In particular, incubation of aortic and brain endothelial cells, hepatocytes, synovial fibroblasts, neutrophils, thymocytes, tumor cells, and renal tubular epithelial cells with 100-μM 4-HNE (this concentration is considered toxic) leads to decomposition of 90-95% of 4-HNE in 3 min in all cell types. In blood serum, the level of 4-HNE taken at near-physiological concentration of 1 μM decreased to 0.1-0.2 μM within 10-20 sec. As primary products of HNE in hepatocytes and other cell types, glutathione–HNE conjugate, hydroxynonenoic acid, and



GST-catalyzed interaction of 4-HNE with reduced glutathione

Scheme 2

the corresponding alcohol of HNE (1,4-dihydroxynonene) were identified. And only 2-8% of 4-HNE was bound with proteins. The rapid metabolism of HNE under oxidative stress (OS) conditions is considered as an important part of the secondary antioxidative defense mechanisms protecting proteins from modification by aldehydic LPO products [36].

An important part of 4-HNE metabolism is early removal of its products from the cell. Data now appear implicating the MRP2 transporter in the removal of GS-HNE metabolites from hepatocytes. The major GS-HNE fraction is transported from the cells into the extracellular space by RLIP76 (Ral-interacting protein) transporter catalyzing ATP-dependent efflux of xenobiotics [37, 38]. Acidic metabolites, in particular 4-HNA, are removed by other transport systems [39].

Thus, analysis of specific features of 4-HNE metabolism indicates that all enzymatic and nonenzymatic pathways of 4-HNE biotransformation play an important role in cell homeostasis, both under normal and under pathological conditions.

4-HYDROXY-2-NONENAL IS A SIGNALING MOLECULE IN CELL METABOLISM

At the end of the 1980s the first reports appeared on the role of LPO products, particularly 4-HNE, in regulation of cell growth and differentiation [40]. Later this was confirmed in a series of studies in which the role of 4-HNE in apoptosis, cell growth and differentiation, stimulation of chemotaxis of neutrophils, and modulation of thrombocyte aggregation was shown [41-46]. At physiological concentrations (0.1-0.3 μM) 4-HNE can act as a signaling molecule. It is implicated in modulation of cell function by activating various signaling pathways and inducing expression of many genes, including "antioxidant" genes [17, 47-51]. Free 4-HNE concentration is 0.3-0.7 μM in blood serum of healthy humans. This level can increase 10-fold and more under OS conditions [52].

4-HNE can form adducts with protein receptors of the cell, thus being implicated in activation of tyrosine kinases such as epidermal growth factor receptor (EGFR). This leads to inhibition of growth via the cascade of EGFR, Shc (SHC-adaptor protein), and ERK (extracellular signal-regulating kinase) activation [53]. At the same time, incubation of cells with 4-HNE is accompanied by decrease in concentration of the intracellular glutathione and GSH/GSSG pool that is associated with induction of proapoptotic mitochondrial signaling pathway via Fas-independent activation of caspase-8, caspase-9, and caspase-3 [54]. The 4-HNE-triggered signal cascade of caspase activation is reflected in a complex of regulatory mechanisms with positive feedback coupled with inhibition of antiapoptotic signals and depends on activity of caspases. Thus, all of these regulatory mechanisms, including activation of Akt-dephosphorylating PP2A (protein phosphatase 2A) and protein tyrosine kinases, are controlled by 4-HNE in a biphasic manner. The latter reacts not only with various components of cell membrane, but also with intracellular elements, thus triggering a signal transduction network. In the aggregate, this results in functional changes and death of the cell [55].

The feature of the regulatory effect of 4-HNE is regarded as depending on its intracellular concentration. However, mechanisms of dose-dependent effect of 4-HNE in the whole body are not clear in detail because most studies have been carried out *in vitro* using cell cul-

tures [56, 57]. In these experiments relatively high concentrations of 4-HNE can induce cell differentiation and apoptosis and influence signaling pathways associated with adenylate cyclase, JNK (c-Jun-N-terminal kinase), protein kinase C (PKC), and caspase 3 [37, 58]. In contrast, low level of 4-HNE is associated with proliferation of some cell types such as aortic smooth muscle cells and K562 (human erythromyeloblastoid leukemia) cells [56, 58-61]. It is likely that there is the narrow limit of physiological concentrations of 4-HNE, and when this limit is exceeded cell differentiation or apoptosis occurs [46]. Decrease in this level leads to cell proliferation. This supposition is supported by proved association between decrease in 4-HNE level due to activity of hGSTA4-4 (a GST isoenzyme) in HLE B-3 cells and their accelerated growth explained by activation of ERK [46].

In K-562 cell line 4-HNE at concentrations of 1-10 μM strongly inhibits both proliferation and differentiation. The same dose-dependent effect of 4-HNE was also revealed in other cell types, particularly HL-60. The treatment of HL-60 cells with 1-10 μM 4-HNE for 1 h inhibited both cell proliferation and expression of the *c-myc* gene. This effect disappeared already in 6-8 h, which, in the authors' opinion, is associated with destruction of 4-HNE that cannot accumulate in the cell [43].

Micromolar concentrations of 4-HNE stimulate apoptosis in various cell types, in particular, they inhibit proliferation and induce death of tumor cells. However, the effect of the same concentration of 4-HNE on cell survival and death differs depending on cell type.

Thus, it is very likely that each cell is characterized by its own "physiological limit" of 4-HNE concentration, which is consistent with its life and proliferating capability. Below this level the cell undergoes uncontrolled proliferation and transformation, and above this level proliferation processes are weakened [43, 46, 57-60, 62].

Data exist in the literature indicating that factors stimulating apoptosis induce LPO processes and are conducive to elevation in levels of 4-HNE and its homologs, α- and β-unsaturated alkenals. It is likely that 4-HNE is a common denominator in apoptosis signaling mechanisms involving these factors. If this is the case, the cells characterized by accelerated metabolism and destruction of 4-HNE should be more resistant to apoptosis [46, 56]. In particular, K-562 line cells subjected to various agents of mild severity (heat, UV irradiation, or incubation with 50-μM H_2O_2 for 20 min) exhibit apoptosis without signs of toxicity. Simultaneously, a transitory increase in 4-HNE level is observed. Thus, all these factors stimulating apoptosis are triggers for 4-HNE production. Two hours after the stimulus, a considerable (20-fold) induction of hGST5,8 and RLIP76 is observed, which is coupled with high production of GS-HNE and its efflux from the cells. No significant induction of antioxidative enzymes or heat shock proteins (HSP70) is observed in this time. It was shown that stress-preconditioned cells compared to control cells possess at least threefold higher capability for GS-HNE production and transport and are also capable of maintaining the physiological level of 4-HNE. Prolonged UVA irradiation, generation of superoxide anions from the xanthine/xanthine oxidase reaction, or the effect of additional 4-HNE lead to significant stimulation of apoptotic processes in control cells compared to stress-preconditioned ones. Resistance of these cells that preliminarily underwent mild OS is explained, in the authors' opinion, by quick elimination of produced 4-HNE from the cells and inhibition of JNK and caspase 3 activation. Thus, experiments with stress-preconditioned cells demonstrate the role of 4-HNE as a common denominator in signaling of apoptosis caused by H_2O_2, $O_2^{\cdot-}$, or UVA [37, 56, 58]. Some authors expect the preconditioning effect to be used in clinical practice, particularly in duodenal ulcer therapy in order to correct the OS state [63].

On the basis of their own and literature data, the authors of [43] concluded that both intensity of LPO and effect of its products on cell function vary depending on the cell cycle phase. Experiment with regenerating liver has shown that low level of LPO is accompanied by maximum activity of thymidine kinases (EC 2.7.1.21), which is coincident with the liver regeneration period. It is likely that these alterations are due to increase in the level of the lipid-soluble antioxidants (AO) that provides the maximum level of DNA synthesis [64]. However, lowered level of aldehyde products of LPO in the proliferating cell might be due to intensification of their metabolism followed by accelerated removal from the cell [65]. In all appearances, it is the mechanism that is activated in normal cells during different phases of the cell cycle, when production of 4-HNE conjugates with glutathione changes in rate depending on both GSH level and GST activity.

Detoxification of 4-HNE occurs with participation of GST isoforms. The 4-HNE level in human tissues is regulated mainly by two minor members of α-class GSTs, hGSTA4-4 and hGST5,8 [28]. They catalyze conjugation of 4-HNE with GSH, and the reaction product is transported into the extracellular space by an ATP-dependent process catalyzed by Ral-interacting protein. So, GST isoforms can modulate the intracellular concentration of 4-HNE implicated in stress-mediated signaling. In particular, expression of specific GST isoenzymes leads to low intracellular level of 4-HNE, which provides the acquisition of limiting resistance of these cells to apoptosis induced by 4-HNE itself, H_2O_2, UVA, and xenobiotic oxidants. A GST-mediated decrease in the level of intracellular 4-HNE influences the growth of cell cultures. The same effect is observed in acceleration of 4-HNE conjugate transport from the cell into the extracellular space. In their turn, inhibitors of enzymes implicated in metabolism of 4-HNE and production of its conjugate with GSH can modulate apoptotic processes induced by tumor necrosis factor alpha (TNF-α) [56]. Thus, the

intracellular concentration of 4-HNE is controlled by coordinated activity of GST isoenzymes (GSTA4-4 and hGST5,8) catalyzing formation of its conjugates with glutathione and transporter 56-kDa Ral-interacting GTPase activating protein RLIP76 implicated in ATP-dependent transport of GS–HNE [66].

A considerable increase in levels of GST A1 and GST A4, two GST isoforms implicated in 4-HNE conjugation, is observed in mice under OS conditions. The level of reduced glutathione is also increased, suggesting additional regulation (sub-regulation) of γ-glutamylcysteine synthetase. Incubation of cells with sub-toxic concentrations (0.5-5.0 μM) of 4-HNE leads to induction of GST A1 and GST A4 enzymatic activities. Their effect might be realized via transcription factor Nrf2 [67].

Incubation of rat cortical neurons with 4-HNE taken at concentrations stimulating apoptosis is accompanied by elevation of binding activity of AP-1 (activator protein-1) with DNA. It was shown that 4-HNE induces phosphorylation of JNK, suppresses Ca^{2+} uptake by mitochondria, and influences permeability of their membranes, which is coupled with activation of caspases. Activated caspases induce JNK, thus stimulating AP-1 DNA-associated protein production. This is the 4-HNE-induced transcription pathway that is considered as modulating the process of cell death [68]. To all appearances, extent of apoptosis, both in normal and neurodegenerative processes, depends on effect of various 4-HNE concentrations on the state of mitochondria [62].

4-HNE at concentration of 10 μM induces apoptosis in human lymphoblastic leukemia cells of line CEM-C7. Interestingly, significantly higher (15-fold and more) concentrations of 4-HNE are necessary for induction of apoptosis in normal human leukocytes. The 4-HNE-induced apoptotic pathway comprises caspases-2, -3, and -8. Caspase-2 is the first caspase cascade-initiating factor that, either directly or via activation of caspase-8, activates caspase-3. On the other hand, stimulation of apoptosis in many cell types is ascribed to overexpression or, sometimes, to downregulation of *c-myc*. In particular, 4-HNE induces downregulation of *c-myc* expression in CEM-C7 cells. Incubation of leukemia cells with 4-HNE for 30 min was accompanied by decrease in *c-myc* expression by almost a third. During this period, the caspase cascade was yet to begin to start, suggesting the proapoptotic role of *c-myc*. Since the used micromolar concentrations of 4-HNE have no effect on apoptosis in normal monocytes, the authors suggest 4-HNE for chemotherapy of leukemia [69].

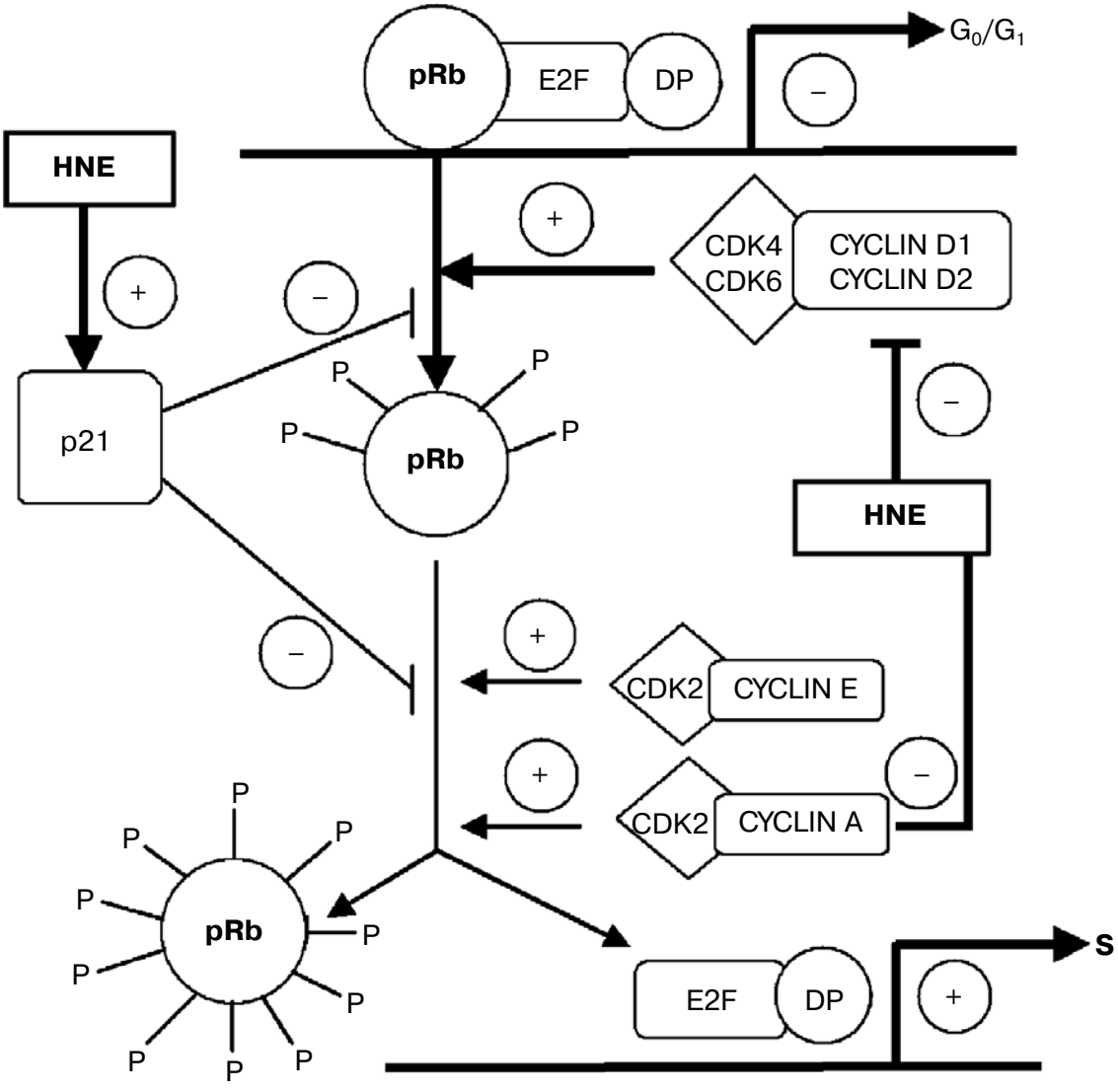
HL cells (which are promyelocytes) are involved in differentiation of the granulocytic population. Their incubation for 7.5 h with 4-HNE is accompanied by induction of the granulocytic differentiation pathway [70]. The differentiation involves biochemical and morphological changes and is accompanied by both *c-myc* and *c-myb* expression inhibition (down-modulation) and cell arrest in G_1-phase [45, 71]. Some increase in G_0/G_1 ratio is observed when HL-60 cells are treated with 4-HNE. This suggests a blocking effect of 4-HNE on progression of HL-60 cells in the cell cycle [44].

The cell progression cycle is known to be controlled by three types of proteins: cyclins, cyclin-dependent kinases (CDKs), and CDK-inhibitors (CKIs). Effects of growth factors and mitogenic signals on cell cycle are coupled with expression of cyclins, whereas deceleration of growth is coupled with expression of CKI and decrease in level of cyclins. There are four cyclin classes. Experiments with HL-60 cells have shown that expression of cyclins D1, D2, and, to less extent, cyclin A decreases following cell treatment with 4-HNE. Other cyclins and CDKs are insensitive to 4-HNE. Low level of cyclins in G_1-phase after cell treatment with 4-HNE might result from their accumulation in G_0/G_1 phase. A low level of S-phase is explained by decrease in the level of cyclin A. Cyclin-CDK activity is controlled not only by amount of cyclins, but also by various CKIs controlling the cyclin-activated kinase activity. There are two structurally distinctive CKI classes. Expression analysis of $p21^{waf1/cip1}$ that belongs to the second CKI class and inhibits many CDKs has shown obvious increase in the level of this inhibitor in 4-HNE-treated HL-60 cells compared to untreated ones [43, 72].

Retinoblastoma protein (pRb) is the main substrate of CDK4/2, whose activity is regulated by inducing activity of cyclins D and A and eliminating the effect of p21. The level of these cyclins is decreased by 4-HNE, which positively influences the level of p21. The state of pRb and corresponding manifestation of its activity depends on the degree of phosphorylation of this protein. Induction of proliferation is accompanied by intense pRb phosphorylation. Antiproliferative stimuli cause the protein hypophosphorylation. In hypophosphorylated state pRb binds to members of the transcription factor E2F family to inactivate them, whereas phosphorylation of pRb results in activation of transcription activity. In mammalian cells E2F is bound with one of the members of the DP-family, proteins required for highly specific and successive binding of E2F with DNA. E2F is a heterodimeric transcription factor that has a specific binding surface for promotor regions of many genes implicated in cell cycle progression. In particular, the E2F protein regulates transcription of *c-myc* by binding to its promotor [73].

Thus, effect of 4-HNE on cell cycle phases G_0/G_1 through S-phase very likely occurs at different levels of the pRb/E2F pathway. One of these levels is decrease in pRb phosphorylation and, probably, inhibition of expression of cyclins D1, D2, and A. The cell cycle progression through G_1-phase is controlled by the functional state of pRb. In phase G_0, pRb protein is hypophosphorylated and so binds to the E2F transcription factor, thus preventing E2F-dependent transcription. One of first steps of transition to the G_1 phase is activation of CDK4 and/or CDK6

kinases due to their mitogen-controlled regulatory subunits, cyclins D1 and D2, which is accompanied by activation of CDK2–cyclin E and CDK2–cyclin A complexes. Phosphorylation of pRb proteins with participation of CDK–cyclin complexes leads to their partial inactivation and makes possible transcription of E2F-controlled genes and progression through the S-phase of the cell cycle. 4-HNE inhibits expression of D1, D2, and A and corresponding activities of CDK4/6 and CDK2. Activity of cyclin–CDK complexes is negatively regulated by $p21^{wafl}$. 4-HNE activates expression of $p21^{wafl}$. Appropriately, the treatment of cells with 4-HNE leads to increase in the level of pRb that binds to E2F. However, the pRb/E2F amount depends not only on phosphorylation degree of pRb, but also on amount of E2F protein that is capable of the binding. Effect of 4-HNE on the pRb/E2F pathway can be realized via regulation of E2F expression. Following treatment with 4-HNE, the E2F4 level decreases for 6, 8, and 24 h. 4-HNE, like other factors possessing modulating effect on differentiation processes, can participate in modulation of expression of distinct members of the E2F family. 4-HNE decreases the level of "free" E2F and simultaneously accelerates the binding of pRb (or its family members) to E2F, thus inhibiting transcription. Thus, 4-HNE breaks E2F transcription activity, being implicated in modulation of a series of genes controlling the pRb/E2F pathway. Finally, 4-HNE can influence the pRb/E2F pathway via induction of p53-independent elevation of $p21^{wafl/cip1}$ expression. All of this leads to decrease in DNA-binding activity of free E2F in 4-HNE-treated cells and correlates with inhibition of *c-myc* expression in HL cells (Scheme 3). Thus, analysis of



Effect of 4-HNE on cell cycle phase G_1 progression [32]

Scheme 3

experimental data has shown an important role of 4-HNE in regulation of cell cycle progression in leukemia cells [43, 71].

A series of studies has shown that 4-HNE possesses antiproliferative effect against various tumor cell cultures. This effect was found against leukemia, human neuroblastoma, and colorectal cancer cells. In this case the concentration of 4-HNE (1 μM) is close to that in nonproliferating cells. Simultaneously, 4-HNE induces apoptosis of colorectal cancer cells [44, 49, 72, 74-77].

In some tumor cells 4-HNE can be implicated in regulation of cell cycle and differentiation of cells. Introduction of 4-HNE into human osteosarcoma (SaOS-2) cells leads to induction of apoptosis in 24 h, which is accompanied by release of cytochrome *c* and activation of the caspase cascade. The treatment of cells with 4-HNE, which is characterized by lowered growth, is accompanied by high degree of cell differentiation. Simultaneously, increase in both alkaline phosphatase and integrin α_5 subunit activity is observed. 4-HNE elevates expression of $\alpha_5\beta_1$ integrins, glycosylated heterodimeric transmembrane adhesive molecules, which are implicated in binding the extracellular matrix with cytoskeleton. 4-HNE-induced expression of integrin α_5 subunit in tumor cells leads to lowering of both their proliferation and metastatic progression [76].

Recent studies have shown that the role of 4-HNE in control of cell replication is associated with its ability to inhibit both telomerase activity and expression of the human telomerase reverse transcriptase (hTERT) catalytic subunit in HL-60, U-937, and ML-1 leukemia cells [75]. Mechanisms of 4-HNE effects on cell growth and differentiation are not understood in detail. One possible mechanism is a 4-HNE effect on expression of microRNAs (miRNAs).

MicroRNAs are a family of phylogenetically inherited low molecular weight noncoding RNAs that act as negative regulators of gene expression at the posttranscriptional level [78]. They play a crucial role in cell development, including differentiation, proliferation, and death [79]. Discovery of this type of RNAs has opened a new approach in studies on biological effects of 4-HNE on cell function. 4-HNE can influence expression of miRNAs. Within the family of miRNAs (470 human miRNAs), only 10 miRNAs demonstrate statistically significant difference between HL-60 cells treated with 4-HNE and untreated ones. Six members (hsa-miR-181a, hsa-miR-199b, hsa-miR-202, hsa-miR-378, hsa-miR-454-3p, and hsa-miR-575) demonstrated lowered expression (downregulated) and four (hsa-miR-125a, hsa-miR-339, hsa-miR-663, and hsa-miR-660) showed elevated expression (upregulated) in comparison with control [80].

It is known that the c-Fos/c-Jun heterodimer comprising AP-1 and belonging to the family of DNA-binding proteins is implicated in regulation of the cell cycle by inducing cyclin D1. Both the platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) are capable of increasing the level of *c-fos* mRNA, which is accompanied by intense proliferation of human carcinoma HeLa cells. The effect of 4-HNE on induction of *c-fos* transcription depends on presence or absence of growth factors. A negative correlation was found between 4-HNE concentration (1-250 μM) and *c-fos* mRNA in absence of growth factors. The authors suppose that its effect is connected with the basal level of *c-fos* promotor, which depends on the nuclear factor NF1 [61]. Pretreatment of cells with 4-HNE (1 μM) in the presence of EGF and PDGF did not influence either cell growth or *c-fos* expression. High concentrations of 4-HNE (100 μM) completely suppressed cell proliferation, although almost twofold increase in expression of *c-fos* mRNA was observed right after addition of the aldehyde. A similar effect of toxic concentrations of 4-HNE on expression of *c-fos* was observed when cells were grown in presence of PDGF. A hypothesis was proposed that 4-HNE is implicated in induction of *c-fos* transcription in different manners depending on presence or absence of growth factors. Cytotoxic concentrations of 4-HNE in the presence of growth factors cause dissociation of elevated expression of *c-fos* during tumor cell proliferation, which is likely associated with blockage of corresponding thiol groups of c-Fos protein [81]. The DNA-binding ability of the c-Fos/c-Jun heterodimer can be modulated due to redox reactions of cysteine residues in DNA-binding domains of both proteins. These cysteine residues can be targets for 4-HNE [68].

The major process regulating amplitude and kinetics of signal transduction, which is required for normal cell life and development, includes timely elimination of active ligand–receptor (particularly, tyrosine kinase receptor) complexes from the cell surface followed by their degradation. This is considered as starting the downregulation process. In contrast, activation of a receptor by H_2O_2 is accompanied by change in its level, thus providing a prolonged receptor response. H_2O_2 stimulates phosphorylation of the receptor tyrosine residues that inhibits its ubiquitination and endocytosis [2]. Thus activation of the receptor by ROS occurs without starting downregulation and can lead to intensified proliferation and tumor development under OS conditions. An association between 4-HNE and downregulation was also shown.

Implication of 4-HNE in regulation of cell growth, differentiation, and apoptosis is of particular importance in tumor development. Ligands of PPAR-β/δ, a member of the nuclear receptor superfamily, inhibit proliferation and induce differentiation or apoptosis in various tumor cells. Since 4-HNE has similar effect, effects of 4-HNE and PPAR-β/δ ligands on these processes were studied [49]. Both the PPAR-γ ligands rosiglitazone and 15-deoxy-prostaglandin J2 (15d-PGJ2) and 4-HNE (1 μM)

inhibit proliferation of human epithelial colorectal adenocarcinoma cells (Caco-2 cell line). The used concentration of 4-HNE is close to that in nonproliferating cells. Extent of the tumor cell growth suppression in presence of 4-HNE is higher than that in the presence of the ligands. The same results were obtained from studies on inhibition of proliferation of leukemia cells [75] and human neuroblastoma SK-N-BE cells [77]. Apoptosis of the cells was induced by 15d-PGJ2, 4-HNE, and to a far lesser degree, by rosiglitazone. Unlike the PPAR-β/δ ligands, 4-HNE in this case had no effect on cell differentiation.

The authors ascribe the proliferation decrease because of PPAR-β/δ ligands and 4-HNE to modulation of *c-myc* gene expression [49]. 4-HNE induces expression of c-Myc protein 8 h after incubation with Caco-2 cells, and complete inhibition of the expression is observed 48 and 72 h after the beginning of the experiment. It is likely that induction of *c-myc* expression because of 4-HNE is associated with induction of apoptosis [82], and subsequent downregulation of *c-myc* expression can depend on distinct inhibition of cell growth. 4-HNE can induce expression of p21 protein, a cyclin/CDK inhibitor, in 48 and 72 h. The PPAR-γ ligands do not possess this feature. Induction of p21 by 4-HNE is coupled with decrease in Caco-2 cell proliferation. When combining the PPAR-γ ligands with 4-HNE, the latter is responsible for elevation of p21 expression [49].

The effect of 4-HNE and 15d-PGJ2 on apoptosis is associated with stimulation of expression of the proapoptotic *Bax* gene [49]. Rosiglitazone, whose effect on apoptosis is insignificant, did not induce expression of the *Bax* gene. PPAR-γ ligands and 4-HNE did not show synergism in Caco-2 cells, in contrast to HL-60 leukemia cells [83]. Thus, *c-myc* is a target for both PPAR-γ ligands and 4-HNE during inhibition of Caco-2 cell proliferation. However, their mechanisms of *c-myc* suppression might differ. Induction of p21 by 4-HNE suggests a putative mechanism of *c-myc* expression via the pRb/E2F pathway, which is also characteristic for HL-60 cells [73].

Among different products of lipid oxidation, only 4-HNE and, to a lesser degree hydroperoxy derivatives, can activate PPAR-β/δ as its agonists. In the authors' opinion, PPAR-β/δ activation by endogenous ligands, particularly 4-HNE, plays an important role in preventing acute and chronic liver injury. Although 4-HNE can covalently bind to cysteine, lysine, and histidine residues of proteins, no covalent bonds were found between PPAR-β/δ and 4-HNE, so its association with 4-HNE is reversible. Thus, 4-HNE acts as modulator of PPAR-β/δ activity [49, 84].

At initial stages of OS, 4-HNE ensures survival of cells due to its interaction with proteins. Actually, this reflects the ability of cells to biotransform highly toxic LPO products into the less reactive ones. This process can explain the ability of 4-HNE to influence metabolic and signal transduction pathways in stress. An implicit ability of 4-HNE to interact with cell signaling molecules was demonstrated on isolated liver astrocytes. Exposure of these cells to 1-10 μM 4-HNE led to formation of 4-HNE adducts of nuclear proteins whose molecular masses corresponded to p46 and p54 isoforms of c-Jun terminal kinase. Translocation of these protein adducts is coupled with increase in the level of c-Jun mRNA, which supports the role of 4-HNE as a signaling molecule under OS conditions. It is likely that this concerns transdifferentiation of astrocytic into myofibroblastic phenotype characterized by proliferation and leading to fibrosis in chronic liver injury [85].

4-HNE can impair signal transduction pathways associated with activation of NF-κB (nuclear factor-κB) transcription factor. Physiological concentrations of 4-HNE inhibit both the NF-κB-mediated release of interleukin-6 (IL-6) from isolated Kupffer cells and transcription activity of NF-κB in Jurkat T, RKO, and H1299 cells. 4-HNE adducts inhibit IκB-kinase β (IKKβ) involved in phosphorylation of IκB (protein inhibitor). In the non-phosphorylated state it remains bound with NF-κB, which abrogates translocation and activation of the transcription factor. 4-HNE seems to form adducts of IKKβ Cys179, the residue providing optimum activity of IKKβ [86, 87].

Recent data suggest the ability of 4-HNE to form stable adducts of specific amino acid residues of tyrosine kinase receptors, in particular, PDGFRβ (platelet-derived growth factor receptor β) from rabbit arterial smooth muscle cells [88]. The effect of 4-HNE is dependent on modification of amino acid residues, possibly, cysteines. It is worth noting that both receptor tyrosine kinases and PDGFRβ contain a cysteine-rich extracellular domain that possibly interacts with 4-HNE. Thus 4-HNE, via proteins prone to adduct formation, can act as biological signal, thus being implicated in activation or suppression of a series of metabolic pathways.

Implication of 4-HNE in regulation of signaling pathways is connected with activation of MAP-kinases. 4-HNE activates all three major MAP-kinase pathways and also atypical protein kinase C and influences the TRE-binding ability of transcription factors (AP-1 binding) [60, 89]. 4-HNE activates AP-1 via the JNK pathway. Hydrogen peroxide is also closely implicated in activation of the JNK pathway [90, 91]. However, it remains unclear whether H_2O_2 acts directly or via generation of 4-HNE. There is a report indicating that 4-HNE can favor stimulation of PKC activity due to release of Nrf2 (NF-E2 related factor 2) from its complex with Keap1 (Kelch-like ECH-associated protein) [46]. Although the activating effect of 4-HNE on p38 MAPK and/or ERK pathways was shown in many studies, mechanisms of this process are not completely understood [60, 62, 85, 88].

4-HNE is implicated in regulation of glutathione metabolism via modulating activities of γ-glutamyl

transpeptidase (GGTP), glutathione synthetase (GS), and glutamate-cysteine ligase (GCL) [92, 93]. Some enzymes can be induced via their direct interaction with 4-HNE [37, 92, 94-96]. GGTP plays an important role in glutathione metabolism by enhancing its turnover and synthesis *de novo* and is implicated in detoxification of xenobiotics via their conjugation with GSH. 4-HNE induces GGTP via expression of GGTP mRNA V-2 [97]. The proximal area of GGTP promotor 5 (GP5) contains several binding surfaces, in particular ARE (antioxidant-response element) or EpRE (electrophile-response element), the manifestation of inducing activity of 4-HNE is associated with. In L2 epithelial cells GP5 promotor activity was induced with 4-HNE. Cell treatment with 4-HNE is accompanied by increase in density of GP5-EpRE protein-binding complex. 4-HNE facilitates the binding of EpRE with Nrf2, a zinc-binding protein that can form heterodimers or other leucine-zinc-binding proteins such as c-Jun and Mafs. This is because 4-HNE forms a conjugate with Keap, a protein that associates Nrf2 with cytoskeleton, thus preventing its translocation into the nucleus. Under physiological conditions, Keap can act as ubiquitin-E3 ligase and provide a rapid Nrf2 cycling due to proteasomal degradation. Dissociation of this complex can occur either via Nrf2 phosphorylation or Keap modification. 4-HNE can be implicated in Nrf2 phosphorylation by PKC resulting in Nrf2 translocation into the nucleus [97, 98]. Thus, induction of GGTP mRNA synthesis by 4-HNE occurs via EpRE/Nrf2 signaling with implication of ERK1/2 and MAPK.

GCL, a key enzyme of GSH synthesis, is composed of two subunits: GCLC (glutamate-cysteine ligase, catalytic subunit) and GCLM (glutamate-cysteine ligase, modifier subunit). 4-HNE triggers a signaling cascade leading to increase in *Gclc* and *Gclm* gene expression with corresponding elevation in contents of both subunits, increase in levels of phosphorylated JNK1 and c-Jun proteins, and elevation of *Gcl* TRE-specific AP-binding activity. This is coupled with increasing GSH content in human bronchus epithelium cells. In fact, 4-HNE is connected with activation of the JNK pathway that, in turn, induces the AP-1 complex via phosphorylation of c-Jun and JunB. Thus, the effect of 4-HNE on GSH synthesis is realized via AP-1, *Gclc* and *Gclm* gene expression, and TRE element localized in promotor regions of many genes [92].

The physiological effect of 4-HNE as a signaling molecule can also be associated with its interaction with thiol groups of proteins implicated in signal transduction. The background is both high reactivity of cysteine and reversibility of 4-HNE–thiol conjugate formation. One can suppose 4-HNE to act as a protector, particularly a protector of proteins. Proteins seem to possess high sensitivity to reactive molecules like 4-HNE [99]. In particular, 4-HNE adducts of neurofilament heavy subunits are formed under normal conditions. Both monomeric actin and actin filaments are modified *in vitro* by 4-HNE at Cys374 due to the Michael addition reaction. Proteins carrying amine groups exposed at the surface of the protein globule possess high sensitivity to reactive α,β-polyunsaturated aldehydes, which prevents toxic effect of the aldehydes and maintains protein function. This is due to plasticity of thus protein domain and its accessibility to toxic aldehydes. In particular, the binding of linear C3 through C9 aldehydes with Cys374 has no effect on actin polymerization because this residue is localized within a very flexible protein area whose covalent modification only induces slight conformational changes without influencing the structure of actin monomer [48].

Because of its prevalence and the high reactivity of the Cys374 residue, actin is regarded as one of the main 4-HNE traps. In lungs, 4-HNE taken at physiological concentrations (1-100 μM) acts as a putative modulator of actin of microfilaments inducing production of Michael adducts in microvascular endothelial cells. This effect was completely abrogated when the cells were pretreated with N-acetylcysteine and mercaptopropionylglycine. All this suggests that the actin Cys374 traps 4-HNE due to the Michael addition reaction. By altering the structure of actin, 4-HNE also influences endothelium permeability and vascular tonus. Incubation of endothelial cells with 4-HNE has shown dose- and time-dependent decrease in lung endothelium cell permeability. The authors suppose that this is due to activation of three MAP-kinase pathways (ERK, JNK, and p38 MAPK) that are implicated in the 4-HNE-mediated modification of actin and thereby influence the barrier function of cells. MAP-kinase inhibitors partially preclude the effect of 4-HNE on actin structure, which suggests their role in regulation of cytoskeleton function [100, 101].

The level of specific 4-HNE-protein derivatives in malignant renal tissue compared to the norm is lowered. A significant amount of protein adduct was found in those tissue areas whose cells actively produced ROS and did not possess adequate antioxidant defense (AOD). In particular, high activity of enzymatic AOD in proximal renal tubules effectively protects cells from ROS, whereas in distal tubules low activity of antioxidant enzymes leads to high level of protein adducts. Actually, the level of protein adducts reflects the physiological role of 4-HNE, because renal cells are characterized by low turnover and, possibly due to formation of 4-HNE–protein adducts, can ameliorate toxicity of LPO products. Thus, the level of adducts differs in different tissues and depends on functional charge [102].

4-HNE is regarded as a regulator of 26S proteasomal degradation of horse liver alcohol dehydrogenase. 4-HNE at concentration of 10 μM modifies two zinc-chelating cysteines of the protein, and this modification serves as a signal of sorts for enzyme ubiquitination and degradation by 26S proteasome. Proteolytic degradation

of proteins, in particular alcohol dehydrogenase, seems to accelerate following formation of adducts with 4-HNE [103].

Thus, the literature data concerning the signal function of 4-HNE implicated in regulation of cell function are quite contradictory, and signaling mechanisms of 4-HNE depending on its concentration in the cell are not well understood.

It is worth noting that, like 4-HNE, some antioxidants have similar effect on cell function. Metabolism of some antioxidants, particularly carotenoids (cyclic retinyl (β-ionone)-containing carotenoids, such as α-, β-, and γ-carotenes and β-cryptoxanthin, acyclic lycopene, cycle-containing canthaxanthin and astaxanthin) yields products that resemble LPO products in chemical nature and biological features. This is due to structural features of their π-electron systems. In polyunsaturated fatty acids (PUFAs) up to six double bonds are separated by methylene groups and in carotenoids up to 11 coupled double bonds form a delocalized π-electron system [104, 105]. The common feature of both systems and simultaneously one of oxidative mechanisms is ability of CH-hydrogen detachment to form a carbon-centered radical with delocalized π-electrons followed by attachment of molecular oxygen with formation of peroxy radical $ROO^{\cdot}$, where R is a PUFA or carotenoid residue. Covalent oxidative destruction of both peroxy radical types leads to formation of a series of cyclic structures, unsaturated and polyunsaturated aldehydes, and their keto- and oxy-derivatives, such as 4-HNE in the case of lipid peroxidation and structurally related 2-methyl-6-oxo-2,4-heptadienal and 4-methyl-8-oxo-2,4,6-nonatrienal (MON) in the case of carotenoid oxidation [106-108].

The products of oxidative destruction of lycopene, primarily MON, induce apoptosis of HL-60 cells. The effect of MON on apoptosis does not depend of ROS and is realized via activation of caspases-8 and -9. Besides, MON inhibits expression of antiapoptotic Bcl-2 and Bcl-X_2 proteins and does not influence the level of proapoptotic Bax protein, suggesting importance of the mitochondrial pathway of apoptosis associated with release of cytochrome *c*. MON-induced apoptosis is mediated by two mechanisms: the mitochondrial pathway and the death receptor pathway. Thus, MON suppresses cell proliferation and induces apoptosis via the same molecular mechanisms as those mediating the 4-HNE covalent interaction with apoptosis-associated proteins [108].

Like 4-HNE, MON and other aldehyde products of carotenoid and PUFA peroxidation can covalently interact with thiol groups and lysine and histidine residues of target proteins. By way of illustration, data can be considered on inhibition of myocardial Na^+,K^+-ATPase that is sensitive to carotenoid oxidation products, namely β-apo-10′-carotenals and retinals, compared to 4-HNE and other PUFA peroxidation products, nonenal and *trans*-2,3-nonenal [109, 110]. IC_{50} values of carotenoid oxidation product mixture is one order of magnitude lower than that of 4-HNE, that is, carotenoids are more potent inhibitors of Na^+,K^+-ATPase than 4-hydroxy-2-nonenal. This fact suggests that peroxidation degradation products of carotenoids at certain concentration can be more toxic than corresponding PUFA peroxidation products. At the same time, aldehyde derivatives of both PUFAs and carotenoids can play a role of autocrine and paracrine agents at extra- and intracellular regulatory levels. De facto, the products of oxidative degradation of PUFAs and carotenoids (the latter possess antioxidant activity and protect PUFAs from peroxidation) run into a unified group of endogenous bioactive metabolite regulators [110].

One can expect that in both classes of polyunsaturated substances each of the above aspects of action – toxic or regulatory – of oxidative destruction products should depend on their metabolic pool, contents, and ratios, and the nature of end-products [111]. Under oxidative stress conditions aldehyde metabolites can accumulate in tissues characterized by high level of carotenoids, and these metabolites, like 4-HNE, step into activation of stress signaling [112].

TOXIC EFFECT OF 4-HNE ON THE ORGANISM

4-HNE is a rather stable and long-living compound that can diffuse in tissues and is highly toxic due to its ability to modify various substances, including proteins, to form derivatives influencing their conformation and functional activity [113-115].

4-HNE damages proteins, mainly by binding with lysine amino groups, cysteine thiols, and histidine imidazole groups [21, 41, 116]. The amino acid residues can be ordered by their sensitivity to 4-HNE as follows: cysteine ⩾ histidine ⩾ lysine [117]. Thus, thiols are most sensitive to 4-HNE, but thiol conjugates are less stable adducts than those of histidine [52]. The modification of histidine protein residues by 4-HNE occurs via addition reaction of the nitrogen atom of the histidine imidazole ring to the region of the aldehyde α,β unsaturated bonds with the formation of stable secondary amines (Fig. 2).

The reaction of 4-HNE with proteins usually results in formation of covalent bonds (cross-linking) giving fluorophores [21]. Lysine dihydropyrrole derivative was identified as the major fluorophore *in vitro*. Two lysine residues interact with 4-HNE to form a Schiff base. Subsequent Michael addition reaction, oxidation, and cycle formation produce dihydropyrrole intra- and intersubunit links (Scheme 4) [118]. Phospholipids containing amine groups can also interact with 4-HNE to form Schiff bases with subsequent cycle formation and production of pyrrole derivatives [119-122].

The reaction of 4-HNE with protein thiols produces thioester derivatives. Note that not only proteins, but also

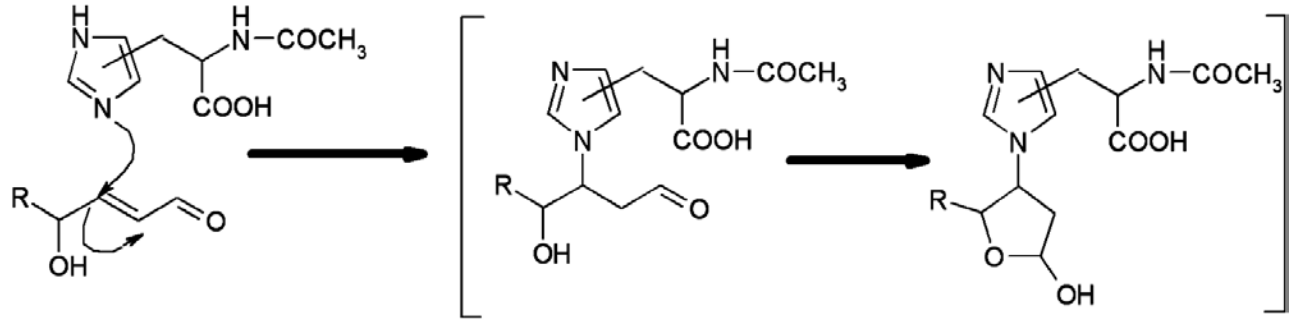


Fig. 2. 4-HNE binding to histidine [116].

low molecular weight compounds containing thiol groups, such as glutathione and cysteine, can react with 4-HNE. Modification of thiols and lysine residues producing stable cyclic derivatives can result in derivative carbonyl products [123, 124].

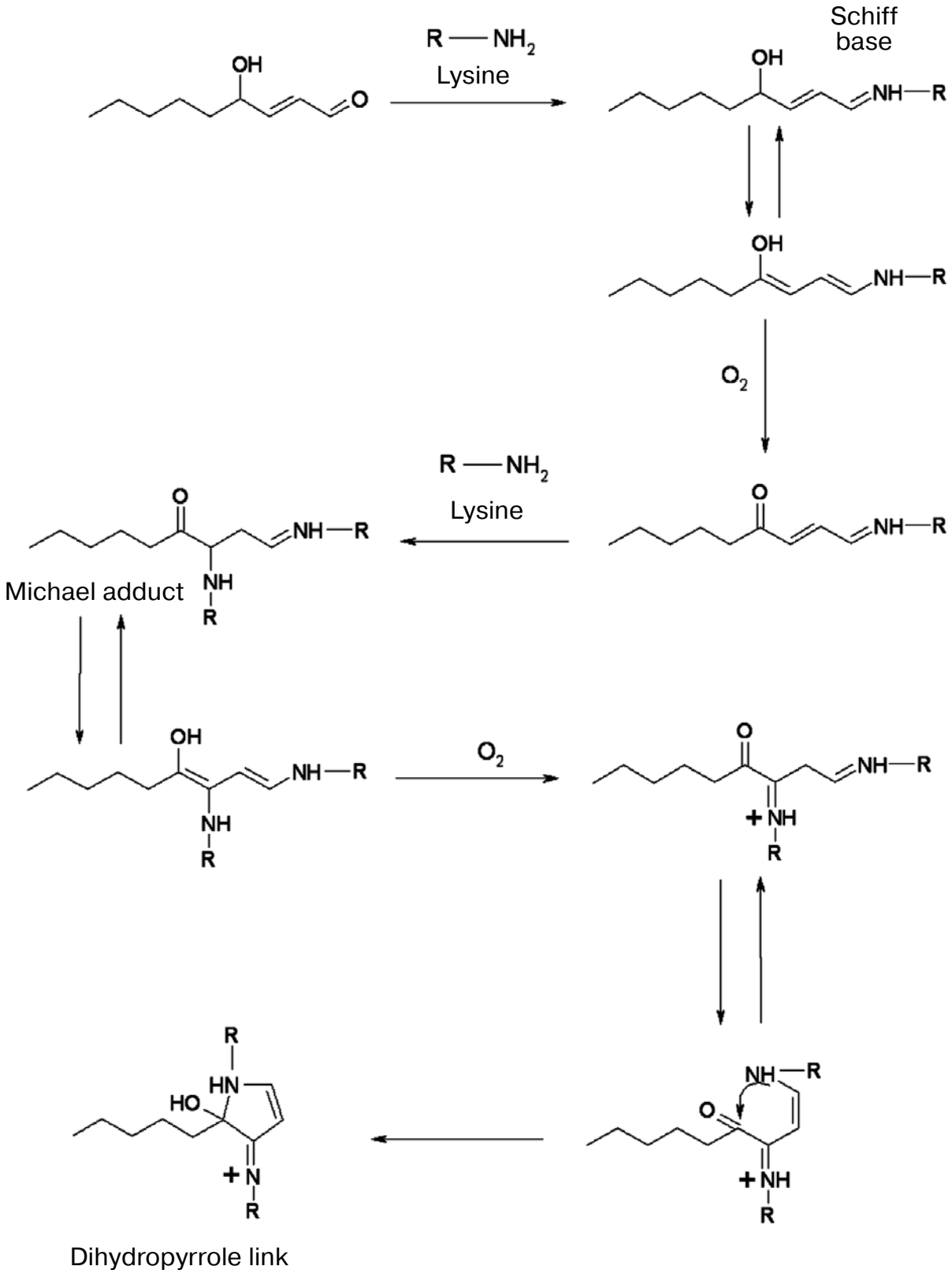
It is the lysine residue modification of certain proteins that plays an important role in pathological processes. So, modification of lysine residues in low density lipoproteins (LDLs) can serve as important indicator of their involvement in arteriosclerosis, and determination of the LDL 4-HNE–lysine adducts can be important for clinical applications [116].

4-HNE-conjugated proteins are found in microglial cells and neurons of ischemic rat brain [125], in damaged spinal cord [126], in sporadic amylotrophic lateral sclerosis [127], and in Alzheimer's disease (AD) [128]. Formation of 4-HNE-conjugated proteins at early stages of neuronal damage resulting in their subsequent death has been demonstrated in experimental studies. Introduction of kainate induced production of 4-HNE-modified proteins in rat hippocampus, whereas these derivatives were absent in normal neurons. The authors believe that 4-HNE realizes its toxic effect at early stages of brain tissue damage induced by kainate [113].

High sensitivity of neurofilament heavy and medium subunits (NF-H and NF-M), the main axonal proteins, to 4-HNE is due to its binding with lysine residues. The intensity of this process increases with aging and is associated with formation of two adduct types: Michael-type products of reversible binding to lysine residues and stable pyrrole-type condensation products of 4-HNE and lysine. Elevated sensitivity of neurofilament proteins to 4-HNE decreases when they become dephosphorylated, which is evidence for the role of protein conformation and phosphorylation in the formation of adducts. The increase in production of 4-HNE adducts is supposed to be associated with the intensive filament protein phosphorylation in AD. NF-H possesses more prominent sensitivity to 4-HNE. The ability of neurofilaments to form adducts with 4-HNE is considered a protective function in relation to axons at the initial stages of OS [129].

Neurodegenerative processes are supposed to be associated with 4-HNE-induced toxicity. Decreasing viability in neurons treated with 4-HNE correlates with increasing protein oxidation extent as judged by the elevated level of carbonyl derivatives and 3-nitrotyrosine, as well as depletion of intracellular GSH pool. The protein–4-HNE complex formation is observed in cell membrane. The authors have demonstrated the protective role of cortical neuron treatment with acetyl-L-carnitine (ALCAR) and α-lipoic acid (LA) under 4-HNE-mediated induced OS accompanied by neurotoxicity. The observed decrease in 4-HNE toxicity was explained by involvement of various signal pathways, including PKG (protein kinase G), ERK1/2 (extracellular signal-regulated kinase), and PI3K (phosphatidylinositol 3-kinase). Activation of the PKG pathway is accompanied by induction of synthesis of antioxidant and antiapoptotic proteins [130].

4-HNE can have an effect on distinct biochemical processes in neurons, such as dephosphorylation and Akt (serine-threonine kinase also known as protein kinase B) deactivation accompanied by dephosphorylation of BAD (proapoptotic factor), mitochondrial depolarization, and impairment of cell permeability. All these factors cause further neurotoxicity and cell death. ALCAR + LA stimulate BAD and ERK1/2 phosphorylation, activity of MnSOD, and increase thioredoxin and Bcl-2 levels, which favors neuronal protection against 4-HNE-mediated induced OS and neurotoxicity. The PI3K/Akt pathway is one of the signaling pathways associated with the protective effect of ALCAR + LA under neurotoxic state caused by 4-HNE-induced OS [131]. Akt plays the key role in the antiapoptotic process associated with PI3K. Phosphorylation of phosphoinositides in membrane by PI3K results in Akt translocation from cytoplasm to cytoplasmic membrane in which Akt is phosphorylated. The phosphorylated form of Akt influences the stimulation of antiapoptotic factors and inhibits proapoptotic factors, for instance, BAD and caspase-9 [132]. β-Amyloid stimulates production of 4-HNE that can initiate a cascade of biochemical reactions, including dephosphorylation, in



Dihydropyrrole cross-linking resulting from interaction of 4-HNE with protein lysine amine groups [10]

Scheme 4

cortical neurons. Akt inactivation is accompanied by BAD dephosphorylation, mitochondrial depolarization, and impairment of membrane penetration, causing neurotoxicity and cell death [41, 133]. Addition of 4-HNE to cultured cortical neurons results in inhibition of receptor G-protein implicated in signal transduction [130, 134]. Note that this data is in line with results obtained in studies on ALCAR and LA effects on rats [135, 136].

Data appear concerning the ability of 4-HNE for specific conjugation with the EAAT2 subtype of glutamate transport system, which leads to deterioration of extracellular glutamate removal from the synaptic cleft [137]. Impaired glutamate transport under OS conditions is accompanied by excitotoxic neuronal death in Alzheimer's disease (AD). β-Amyloid elevates 4-HNE-binding with the EAAT2 transport system. GSH prevents the 4-HNE-mediated impairment of glutamate and glucose transport in rat cortical synaptosomes [138]. The 4-HNE-dependent impairment of transport in AD was also found in lymphocytes. Glutamate transport in cultured fibroblasts of AD patients compared to healthy humans is more sensitive to damaging effect of 4-HNE. Impairment of glutamate transport is observed 2 h after incubation, and its extent depends on 4-HNE concentration. The most pronounced decrease in glutamate transport was observed at 4-HNE concentration of 50 μM. Unlike the brain, EAAT1 and EAAT3 rather than EAAT2 glutamate transport subtypes are inhibited in fibroblasts. So, dysfunction of the glutamatergic system in AD is connected with OS with the significant involvement of 4-HNE [129].

4-HNE toxicity depends on the ability of cells to biotransform it into nontoxic products. Isolated hepatocytes are highly resistant to 4-HNE. This is due to high activity of redox processes involving 4-HNE and enzymatic conjugation with glutathione [22]. Fibroblasts are more sensitive to 4-HNE because of their low ability for its detoxification. Production of GS-conjugates is an important process not only in 4-HNE detoxification in liver and heart, but also in regulation of cell response to aldehyde production in LPO [30, 39, 139].

Formation of aldehyde–protein adducts inhibits many enzymes such as glyceraldehyde-3-phosphate dehydrogenase, hexokinase, aldolase, lactate dehydrogenase, glucose-6-phosphatase, 5′-nucleotidase, DNA-polymerase, and adenylate cyclase. This leads to impairment of metabolic pathways providing synthesis of nucleic acids and proteins, tissue respiration, glycolysis, Ca^{2+}-cascade, etc., which reflects on functional activity of cells [56]. For example, incubation of *Leuconostoc mesenteroides* glyceraldehyde-3-phosphate dehydrogenase with 4-HNE inhibits its enzymatic activity, the extent depending on both 4-HNE concentration and duration of incubation. The concentration of carbonyl groups increases in parallel. The treatment of the enzyme with 2 mM 4-HNE for 12 h at 36°C modified 74% of cysteine, 16% of lysine, and 50% of histidine residues of the protein [123].

In ischemia/reperfusion of rat myocardium, 4-HNE is implicated in inactivation of mitochondrial cytochrome *c* oxidase. A similar effect was found *in vitro* with purified enzyme [140]. 4-HNE exhibits toxicity in cardiovascular and pulmonary disorders [18]. It elevates cell permeability, which might be associated with change in thiol redox status. This leads to modulation of cellular signaling pathways and, as a result, to impairment of endothelial barrier function. Incubation of microvascular endothelial cells from bovine lung with 4-HNE is accompanied by generation of ROS, exhaustion of intracellular glutathione pool, and alteration of cell–cell adhesion. Integrins play an important role in 4-HNE-mediated endothelial cell permeability, which is mediated by formation of Michael adducts of integrins α5 and β3 resulting in contraction of their binding surface. All these changes are accompanied by a profound impairment of barrier function of endothelial cells in pulmonary diseases, cardiac infarction, and stroke [101].

When studying oxidative modification of LDLs, the oxidation mechanism of their protein part is of interest [141]. MDA and 4-HNE generation is accompanied by formation of Schiff bases and/or Michael reaction with specific amino acid residues [41, 142]. Lysine residues actively participate in LDL modification resulting in generation of immune complexes and elevation of activity of macrophages [143]. 4-HNE-modified LDLs are accumulated in macrophages as a high molecular weight fraction stable to lysosomal proteolysis. The oxidative modification of LDLs proved to be associated with high molecular weight apolipoprotein B-100 fraction due to intra- and intermolecular cross-linking. This makes the protein stable to proteolysis [142]. One can propose that peroxy radicals formed under membrane lipid oxidation, as well as LDLs, participate in oxidation of the protein parts. Formation of carbonyl derivatives of apolipoprotein B-100, amino acid derivation, and protein fragmentation are due to the action of radical products of lipids and aldehydes, including 4-HNE [144-147].

4-HNE induces covalent modification of epithelial fatty acid-binding protein (E-FABP) in experiments *in vivo* and *in vitro* due to the modification of Cys120 [148]. This removes reactive aldehydes from the medium. E-FABP is regarded as a special antioxidant trap under OS conditions. Tissues and organs sensitive to OS, such as retina, lung, lens, and tongue, are found to contain high levels of E-FABP. The binding of E-FABP to 4-HNE neutralizes toxic effect of the aldehyde and, to some extent, protects other proteins from oxidative destruction [149, 150].

Incubation of 4-HNE with α-synuclein results in its covalent modification with formation of Michael adducts. Increase in β-cross-linking extent is observed, as well as formation of densely packed oligomers having low dissociation rate and high stability. These oligomers, being added to middle brain cell culture, induce neuro-

toxicity and impair integrity of dopaminergic and GABAergic domains [151].

Oxidation of intracellular proteins with implication of 4-HNE has been revealed in carcinogenesis [152, 153]. Formation of lipofuscins during aging and neurodegenerative diseases is associated with modification of biomolecules by aldehyde LPO products. 4-HNE is implicated in stress signaling via inhibition of the NF-κB/Rel system and c-JNK activation [50, 60, 154]. Inhibition of Na^+,K^+-ATPase by 4-HNE is due to an interaction with cysteine and lysine residues of the protein [155].

Modification by 4-HNE of rat retinal proteins is observed under photooxidative stress, the severity increasing 1.5-fold compared with control. 4-HNE rapidly reacts with histidine, cysteine, or lysine residues of proteins to form stable Michael adducts of hemiacetal structure. Nine 4-HNE-modified retinal proteins were identified at early stage of stress-stimulated degeneration of retina. Voltage-dependent anion channels, aldolase C, enolase 1α, and heterotrimeric G-proteins were modified by 4-HNE. Interaction of G-protein subunits with 4-HNE can lead to reduction of photoreceptor function. 4-HNE also modifies serum albumin. Appearance of albumin in retina is assigned to increased permeability of the blood–retinal barrier. 4-HNE is supposed to damage molecular associations not only in sites of the initial oxidative stress, but also in distant areas, because aldehydes are characterized by significantly longer half-life times than reactive free radicals [119, 134].

4-HNE-modified proteins after the cross-linking reactions accumulate in cells due to the inhibition of proteasomes, thus inhibiting turnover of other proteins in tissues. So, 4-HNE-modified amyloid β participates in formation of senile plaques due to the formation of cross-links inhibiting activity of proteasomes in AD. Neither 4-HNE (100 μM) nor amyloid β possess this ability. Chymotrypsin-like activity of 20S is inhibited due to modification by 4-HNE. 20S proteasomes become more sensitive to oxidative modification during aging. 20S β-subunit is shown to become glycosylated, 4-HNE-modified, and ubiquitinated during aging. Retardation of NF-κB induction in T-lymphocytes is associated with a decrease in chymotrypsin-like proteolytic activity during aging [156, 157].

4-HNE level increases 3-10-fold in patients with rheumatoid arthritis, system sclerosis, lupus erythematosus, and chronic renal injury [36].

Gastrointestinal endoscopy of patients with gastric ulcer and duodenal ulcer has revealed accumulation of 4-HNE in epithelial tissue cells, both in the cytoplasm and nucleus. Since 4-HNE is highly toxic, its accumulation can cause further malignization of tissue. Interestingly, 4-HNE is present in gastric mucosa in healthy subjects, but only in cytoplasm of the cells, which can be explained, in the authors' opinion, by a physiological role of 4-HNE in granular epithelium. Normal nuclei are protected from the accumulation of LPO products in nuclear and perinuclear space by potent antioxidant defense (AOD). Besides, unlike cytoplasm, they do not accumulate oxidized proteins due to high proteolytic activity of nuclei. The authors suppose that the accumulation of these products in pathology is associated with the OS condition and depletion of AOD. Accumulation of 4-HNE-modified proteins associated with nuclear membrane is observed. This might be due to inhibition of the proteasome system by high levels of intracellular 4-HNE. All these processes can favor further tissue malignization [158].

Autoimmune diseases are accompanied by enhancement of ROS production, intensification of LPO, and formation of aldehyde-modified proteins. Corresponding antibodies were raised against these modified proteins, which makes it reasonable to consider them as markers of various autoimmune diseases [159, 160]. Aldehyde adducts of proteins are immunological triggers for T-lymphocyte activation. They cause extended proliferation of $CD4^+$ T-cells that are associated with autoimmune inflammation. Activation of Th1 (T helper cell) population is accompanied by secretion of anti-inflammatory cytokines, such as IL-2, interferon-γ, and TNF-α, stimulating macrophages. The latter execute phagocytosis coupled with ROS production. The effect of aldehyde-modified proteins in autoimmune states is associated with induction of Th1-cellular activity. Thus, a distinct association has been shown between OS and induction of autoimmune reaction of the organism, and the role of proteins modified with aldehydes (MDA and 4-HNE) has been confirmed in activation of T-cells and differentiation of Th1 favoring autoimmune response [160].

Administration to rats of major bacterial lipopolysaccharide enterotoxin results in OS development and elevation of 4-HNE level in intestinal mucosa. This was first found *in vivo*, using a rat model of intestinal inflammation, and *in vitro* 4-HNE influences bacterial activity and causes polymerization of immunoglobulin A (IgA). Modification of human and rat IgAs by 4-HNE (5 μM through 5 mM) decreases their antibacterial activity. Both physiological and toxic 4-HNE concentrations inhibit IgA activity. At the same time, 4-HNE induces genes associated with inflammation, particularly with cyclooxygenase-2, stimulates migration/activation of leucocytes and macrophages, and activates phospholipase C in inflammatory process [161, 162].

Expression of 4-HNE genotoxicity varies depending on cell type, which is probably due to different metabolic level and different predisposition to differentiation. For instance, hepatocytes and brain microvascular endothelial cells possessing higher metabolic activity are very sensitive to 4-HNE at concentrations of 0.1 through 10 μM. Incubation of these cells with 1 μM 4-HNE for 3 h leads to increase in number of micronuclei, and increase of 4-HNE concentration to 10 μM is accompanied by chromosomal aberrations [163].

Toxic effect of 4-HNE on DNA compared to proteins manifests at lower (1-10 μM) concentrations, because formed protein conjugates undergo degradation more rapidly. Experiments *in vitro* have shown that both mutagenicity and genotoxicity of 4-HNE result from its interaction with guanine bases of DNA [164]. Two pathways associated with 4-HNE mutagenicity are considered. The first implies interaction of 4-HNE with guanosine in DNA, whereas the second involves 4-HNE oxidation into epoxide followed by reaction with guanosine residue to form the 1,N^2-etheno-guanosine adducts [21]. Some of these adducts have been found in human and rat DNAs, which points to their endogenous nature [165]. 4-HNE-adducts of 2′-deoxyguanosine-5′-monophosphate can be implicated in *p53* gene mutations in Wilson disease and carcinogenesis [166]. As mentioned above, GSH is the major 4-HNE trap and key element of its detoxification. Production of 4-HNE–GSH conjugate fourfold exceeds that of DNA adducts, suggesting the crucial role of GSH in protection of DNA.

Thus, the mechanisms of 4-HNE effects on cell functions, such as proliferation, differentiation, apoptosis, and regulation of gene expression, are now thoroughly studied. It is worth noting that the majority of data has been obtained from experiments on cell cultures, which creates certain difficulties in estimation of effects of 4-HNE on the whole body. To all appearances, each different cell culture possesses its individual physiological 4-HNE level that, along with other intra- and extracellular regulators, supports normal cell functions. Under pathological conditions, excess 4-HNE generated during enhanced LPO demonstrates toxic effect connected with oxidative destruction of proteins, nucleic acids, and carbohydrates and also with modulation of gene expression. 4-HNE is regarded as one of the triggering mechanisms of apoptosis in neurodegenerative disorders. It is likely that in the future signaling and modulating effects of 4-HNE on metabolic processes in the organism will underlie its application as a therapeutic agent in a number of pathologies, particularly in cancer.

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