



Damage of DNA and proteins by major lipid peroxidation products in genome stability

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Manuscripts

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Warsaw, 13 January 2012

8 Prof. Michael Davies
9 **Editor,**
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12
13

Re Ms: GFRR-R-2011-0362

14
15 Dear Prof. Davies,
16

17 Thank you very much for considering our manuscript GFRR-R-2011-0362 for publication in *Free*
18 *Radical Research*
19

20 **"Lipid peroxidation induced damage of DNA and proteins in genome stability"** by Winczura
21 et. al.
22

23
24 We would like to thank the reviewers for their valuable comments. Enclosed, please find the revised
25 version, which includes answers to all questions raised by the reviewers. We changed the text and
26 table presentation according to reviewers recommendations. All changes are marked in the text in
27 red.
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29 Sincerely yours
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31 Barbara Tudek
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4 Answer to comments of **Reviewer 1:**
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6 *1. There are a number of abbreviations used in the text which it would be helpful to add to the*
7 *Abbreviations list at the beginning of the document (e.g. M1dG, M1dA, AcrdG, CrodG, BER,*
8 *NER, MDA, HNA, DHN, NSCLC).*
9

10 We included suggested abbreviations as well as added several new to the List of
11 Abbreviations.
12

13
14 *2. Page 4, 3rd line of text. There should, presumably be a closing) and full stop after*
15 *“[formula 1]”.*
16

17 Corrected.
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19
20 *3. Page 4: it should perhaps be also noted that lipid hydroperoxides can be directly reduced,*
21 *in a two-electron process, to the corresponding alcohols by species such as glutathione*
22 *peroxidase-4.*
23

24 We included this suggestion into the final text on p. 5. bottom.
25

26
27 *4. Page 4, second paragraph. The second and third lines may be clearer if reworded as*
28 *follows ”undergoes cyclization to a cyclised peroxy radical [formula 5], which can*
29 *undergo further reactions to form unstable peroxides and a series of unstable oxidised fatty*
30 *acid products (Fig. 1. [formula 6 and 7])*
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32 We simplified this paragraph according to the suggestion of the reviewer.
33

34
35 *5. Page 4, second paragraph, last line: converts the lipid peroxy radicals into a relatively*
36 *stable lipid hydroperoxides [7,10].*
37

38 Corrected
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40
41 *6. Page 5, section 3: it would be very helpful for the reader if data on the approximate levels*
42 *of HNE, peroxides and related species were given. This is of critical importance in evaluating*
43 *cell studies where these materials are added in, often at huge levels that are clearly not*
44 *pathologically relevant. It is important that new workers in the field, do not succumb to using*
45 *levels that are clearly inappropriate.*
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48
49 On p. 6 we added a paragraph about the levels of lipid hydroperoxides and TBARS in human
50 blood plasma and lymphocytes.
51

52
53 *7. Page 6. This reviewer is not a fan of single sentence paragraphs. To my mind it would be*
54 *better if the various small paragraphs written here were linked together in a single*
55 *paragraph.*
56

57 We linked together small paragraphs previously slightly changing composition of this part of
58 the final text.
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4 8. Page 7, line 7 of section 4: export of this complex from the cell...

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6 Corrected

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10 9. Page 10, penultimate line: the sentence "It is suggested that they derive from HNE..."
11 needs a reference.

12
13 Reference added.

14
15 10. Page 14, section 8: as above, some absolute estimates as to the levels / abundance of
16 these materials in pathological specimens would be very helpful and useful (fold increases are
17 interesting – as given in Table 1, but the absolute values are more valuable and useful).

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19
20 Absolute values of ethenoadducts levels found in pathological specimens were included as a
21 new column to the Table 1.

22
23 11. The reference section needs to be re-formatted in the style of the journal (e.g. list all
24 authors, text not in bold, etc.

25
26 The reference section was reformatted.

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31 Answer to comments of **Reviewer 2:**

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34 1. The authors mostly presented about HNE and MDA, even though the title is "Lipid
35 peroxidation ---". There are a lot of lipid peroxides, and each one gives different adduct. It
36 may be better to improve the title.

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40 We agree that there are plethora of LPO products, and we were unable to describe all of them.
41 However, the consequences of the major LPO products were described. That is why we
42 changes the title to the following:

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45 "Damage of DNA and proteins by major lipid peroxidation products in genome stability"

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48 2. Keywords in the first page are not same as in the title page.

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50 Corrected

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54 3. Figure 6: The legend should be "by malondialdehyde products" but not "by LPO
55 products".

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57 Legend was changed to "The major exocyclic DNA adducts"

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6 **Damage of DNA and proteins by major lipid peroxidation products in**
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8 **genome stability**
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10
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44 Key words: HNE; DNA repair; lipid peroxidation; DNA-adducts
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48 Abbreviations: Acr-dG, acrolein deoxyguanosine adduct; BER, Base Excision Repair; Cro-
49 dG, crotonaldehyde deoxyguanosine adduct; DHN, 1,4-dihydroxy-2-nonene; εA, 1,N⁶-
50 ethenoadenine; εC, 3,N⁴-ethenocytosine; 1,N²-εG, 1,N²-ethenoguanine; N²,3εG, N²,3-
51 ethenoguanine; EH, 2,3-epoxy-4-hydroxynonanal; ER, endoplasmic reticulum; Et-ethyl
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4 group; HNA, 4-hydroxy-2-nonenic acid; HNE, *trans*-4-hydroxy-2-nonenal; LPO, lipid
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6 peroxidation; M1dA, N⁶-(3-oxopropenyl)-2'-deoxyadenosine; M1dG, pyrimido-[1,2 α]purine-
7
8
9 10(3H)-one-2'-deoxyribose; MDA, malondialdehyde; Me-methyl group; NER, Nucleotide
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12 Excision Repair; NSCLC, non-small cell lung cancer
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For Peer-Review Only

Abstract

Oxidative stress and lipid peroxidation (LPO) accompanying infections and chronic inflammation may induce several human cancers. LPO products are characterized by carbohydrate chains of different length, reactive aldehyde groups and double bonds, which make these molecules reactive to nucleic acids, proteins and cellular thiols. LPO-derived adducts to DNA bases form etheno-type and propano-type exocyclic rings, which have profound mutagenic potential, and are elevated in several cancer-prone diseases. Adducts of long chain LPO products to DNA bases inhibits transcription. Elimination from DNA of LPO-induced lesions is executed by several repair systems: base excision repair (BER), direct reversal by AlkB family proteins, nucleotide excision repair (NER) and recombination. Modifications of proteins with LPO products may regulate cellular processes like apoptosis, cell signaling and senescence. This review summarizes consequences of LPO products presence in cell, particularly 4-hydroxy-2-nonenal in terms of genomic stability.

1. Introduction

Living organisms undergo permanent exposition to damaging agents which may arise from environmental and endogenous processes. Damaging agents react with all cellular components, including proteins, lipids and nucleic acids. It has been estimated that every day approximately 20 000 DNA lesions originating from endogenous sources are formed in every cell of the human organism. Several mechanisms participate in the generation of DNA modifications, namely deamination, spontaneous hydrolysis of the N-glycosidic bond, alkylation and activity of free radicals, which include reactive oxygen (ROS) and nitrogen species (RNS), and lipid peroxidation (LPO) products [1-3]. However, ROS, RNS and, LPO products play a dual role in cells. At low or moderate concentrations they are mediators of specific physiological processes. High concentrations can be detrimental to all components of the cell. Overproduction of oxygen species combined with insufficiency in protective mechanisms of anti-oxidative defense is defined as oxidative stress [4, 5].

DNA damage caused by free radicals is probably the most heterogeneous group of DNA lesions due to the wide variety of deleterious molecules produced in cells in the conditions of oxidative stress. Free radicals can be generated by irradiation (X rays, γ -rays, UV light), drugs or heat; however, most of them are formed in normal metabolic processes that occur in every cell [4-6].

This paper will focus on lipid peroxidation, interaction of LPO products with DNA and proteins and biological consequences of these processes.

2. Lipid Peroxidation

A growing body of evidence suggests that many of the detrimental cellular effects observed under oxidative stress conditions are mediated by the products of lipid degradation [7]. Particularly susceptible to peroxidation are polyunsaturated fatty acids (PUFA) as they

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3 possess a number of double bonds. Lipid peroxidation occurs in three phases, initiation,
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5 propagation and termination [8]. In the initiation phase an oxidant abstracts a hydrogen atom
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7 from a C-H bond of lipid to generate carbon-centered lipid radical (Fig.1 [formula 1]). In the
8
9 propagation phase carbon-centered radical reacts with O₂ to form peroxy radical (Fig. 1
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11 [formula 3]). Peroxy radical may further react with another molecule of PUFA and abstract a
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13 hydrogen atom from the fatty acid chain. This feature leads to propagation of the chain
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15 reaction of lipid peroxidation. As a result, a single initiation reaction can generate hundreds of
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17 lipid peroxides of different structures. For example, if the peroxy radical exists at one of the
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19 two ends of the double-bond (Fig. 1 [formula 2]), it is reduced to hydroperoxide (Fig. 1
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21 [formula 4]). Conjugated diene hydroperoxides (Fig. 1 [formula 4]) are stable in the absence
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23 of metals. Metal complexes and metalloproteins abundant in cells can reduce all fatty acid
24
25 hydroperoxides to alkoxy radicals, which subsequently undergo multiple chemical reactions
26
27 to generate broad range of products, depending on the chemical structure of the precursor
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29 lipid [9].
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34 If the peroxy radical is at the internal position in the fatty acid chain (Fig. 1 [formula
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36 3]) it undergoes cyclization to a cyclised peroxy radical (Fig. 1 [formula 5]), which can
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38 undergo further reactions to form a series of unstable oxidized fatty acids (Fig. 1 [formula 6
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40 and 7]). These fatty acids give rise to final products, like isoprostanes and malondialdehyde
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42 (MDA) [9]. LPO is terminated when the chain reaction is interrupted by a reaction of lipid
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44 radical with another lipid radical or lipid peroxide with a molecule of antioxidant, which
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46 provides the hydrogen atom that converts the lipid peroxy radicals into a relatively stable
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48 lipid hydroperoxides [7, 10].
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52 Lipid hydroperoxides may undergo further reactions and rearrangements. They can be
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54 directly reduced to the corresponding alcohols by e.g. glutathione peroxidase GPx4, which
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56 was reported as a major cellular enzyme dedicated for lipid hydroperoxides scavenging [11,
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3 12]. Alternatively, lipid hydroperoxides give rise to several other groups of molecules that
4 include smaller derivatives of the original molecule. These short chain reactive aldehydes are
5 relatively stable and can diffuse throughout the whole cell attacking other biomolecules. Short
6 chain reactive aldehydes are classified into the following families: 2-alkenals (e.g. acrolein,
7 crotonaldehyde, 2-hexenal), 4-hydroxy-2-alkenals (e.g. 4-hydroxy-2-hexenal - HHE, 4-
8 hydroxy-2-nonenal - HNE) and ketoaldehydes (e.g. malondialdehyde - MDA, glyoxal, 4-oxo-
9 2-nonenal - ONE) [7] (Fig. 2). Multiplicity of LPO products is tremendously augmented by
10 the existence of stereoisomers of several of these compounds.
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20 Concentrations of reactive aldehydes usually vary depending on the tissue analysed,
21 health status and age of examined organism. In solid tissues the level is usually an order of
22 magnitude or more higher than in blood plasma [20]. In blood serum of breast cancer women
23 the level of plasma lipid hydroperoxides (LH) ranged from 0.12 to 1.85 μM whereas plasma
24 TBARS (Thiobarbituric Acid Reactive Substances, well-established method for monitoring
25 lipid peroxidation) ranged from 3.09 to 6.84 μM [13]. TBARS in healthy children ranged
26 from 1.22 to 1.36 μM [14] whereas in another study TBARS in healthy male subjects ranged
27 from 0.5 to 2.2 μM (average $1.2 \pm 0.3 \mu\text{M}$) in sera whereas average level of lipid
28 hydroperoxides was $3.9 \pm 1.5 \mu\text{M}$ (range 1.9- 6.9) in sera [15]. The other study determined the
29 levels of MDA in lymphocytes obtained from healthy subjects grouped according to the age.
30 The average MDA level was $12.35 \pm 0.37 \text{ nmol/mg protein}$ in the group of young persons
31 (11-20 years old) and increased with age reaching the highest average value of 33.63 ± 0.42
32 nmol/mg protein in the group of elderly persons (51-60 years) [16].
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55 3. HNE as a prevalent LPO product and its reactivity

56 Among numerous products of lipid peroxidation two major and most extensively
57 studied are malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). One of the possible
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3 pathways of MDA formation is presented in Fig. 1. The best known consequences of MDA
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5 presence in cells are MDA interactions with nucleic acids and induction of mutations
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7 (described below). However, best described in respect of protein modification, changing gene
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9 transcription, and cell signaling is HNE. Although probably all LPO products interact with
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11 proteins and may affect cell metabolism, knowledge of the resulting consequences is mostly
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13 based on reactions of HNE.
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16 HNE is generated from ω 6 PUFA, mainly during non-enzymatic peroxidation of
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18 linoleic acid and arachidonic acid. In a study using 9 and 13 hydroperoxides of linoleic acid
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20 (13-hydroxyperoxy-octadecenoic acid (13(S)-HPODE) and 9-hydroxyperoxy-octadecenoic
21
22 acid (9(R)-HPODE)) as initial material, it was shown that two distinct mechanisms may lead
23
24 to the formation of HNE. Hydrogen abstraction from C8 of 13(S)-HPODE and subsequent
25
26 cleavage of the resulting 10, 13-dihydroperoxide between C9 and C10 gives 4(S)-HPNE (4-
27
28 hydroperoxy-2-nonenal). Cleavage of 9(R)-HPODE forms 3Z-nonenal, which is a precursor
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30 of 4(R,S)-HPNE. Subsequent simple reduction step of 4-HPNE leads to HNE formation (Fig.
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32 3) [7, 17].
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36 Apart from uncontrolled radical-derived pathway of HNE formation, a certain amount
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38 of this compound may be generated *via* enzymatic reactions. Two most important enzymes
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40 are lipooxygenases (LOX) and cyclooxygenases (COX), which oxidize arachidonic acid to
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42 form mediators of inflammation, prostaglandins, prostacyclins or leukotrienes. The finding
43
44 that 15LOX isoform oxidizes linoleates exclusively to 13(S)-HPODE, a precursor for HNE,
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46 has led to inclusion of these enzymes in the group of potential factors enhancing LPO [18,
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48 19].
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51 HNE is an amphiphilic compound with three functional chemical groups, which
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53 determine its reactivity. Interplay of the double CC bond, carbonyl group (CO) and hydroxyl
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3 group (OH) provides partially positive charge at carbon 3 and carbon 1, which are the sites of
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5 nucleophilic attack by other compounds (e.g., thiols or amino groups).
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7 HNE can be involved in two types of chemical reactions. It may undergo Michael
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9 addition reaction with biomolecules containing amino and/or thiol groups like cysteine,
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11 lysine, the imidazole group of histidine or glutathione (Fig. 4), as well as it can form Schiff-
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13 base with primary amines (e.g., lysine) (Fig 5) [10]. The latter reaction is competitive with
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15 Michael addition. Nevertheless, within cells, HNE may contribute to cross-linking of proteins.
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17 The reaction of Schiff base formation between HNE and lysine of a particular protein
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19 followed by the second reaction of Michael addition with a lysine of another protein results in
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21 binding of two proteins [10]. Reactivity of HNE with proteins and nucleic acids, which are the
22
23 primary targets for HNE, determines its biological effects in cell. HNE forms adducts to side
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25 chains of Cys, His and Lys in proteins. Among them the most reactive with HNE is Cys,
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27 which undergoes HNE addition two and three orders of magnitude more efficiently than His
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29 and Lys, respectively [20]. However, the exact site of modification of proteins by HNE is
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31 determined not only by the reactivity, but also by additional factors like polarity of
32
33 microenvironment, tertiary structure of protein or accessibility of the side chain to HNE.
34
35 There is a plethora of data documenting protein modification by HNE *in vitro* and *in vivo*.
36
37 HNE targets a variety of oxidoreductases, transferases, hydrolases, lyases, kinases, ion
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39 channels and many other proteins. In general, HNE adducts to proteins diminish their activity,
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41 although some examples of activation are also known [20].
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50 **4. Biological effect of HNE**

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52 Intracellular concentration of HNE may range from submicromolar, usually 0.1-3 μM ,
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54 in blood plasma under physiological conditions, up to 5 mM under conditions of oxidative
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56 stress in cellular compartments adjacent to membranes. Concentration of HNE in cell is
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3 mostly regulated by a coordinated function of glutathione-S-transferases (mainly by GSTA4-4
4 and hGST5.8 isoforms) and the RLIP76 transporter. Glutathione-S-transferases (GSTs)
5 catalyze conjugation of HNE to glutathione (GSH), whereas RLIP76 is engaged in ATP-
6 dependent export of this complex from the cell [21]. HNE-GSH transfer is the most rapid and
7 effective way of HNE neutralization, but HNE can also be metabolized by aldehyde
8 dehydrogenase to corresponding carboxylic acid – HNA (4-hydroxy-2-nonenic acid), and
9 alcohol dehydrogenase to corresponding alcohol – DHN (1,4-dihydroxy-2-nonenol) [22]. The
10 proportion between these three metabolites in rat hepatocytes was 6:4:1 for HNE-GSH: HNA:
11 DHN respectively [23].
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23 Although cells are equipped with tools that prevent uncontrolled action of HNE, under
24 conditions of oxidative stress defense mechanisms seem to be insufficient to maintain cellular
25 homeostasis. HNE was shown to be a ubiquitous molecule that could very easily diffuse along
26 all cellular compartments in a much more effective way than “oxygen mediators”, like
27 hydrogen peroxide that acted mainly in the cytosol [24]. HNE at 0.1-10 μM , close to
28 physiological concentrations in blood plasma, stimulates cellular proliferation, reduces
29 differentiation and activates cytoprotective response. It activates JNK and p38 kinases which
30 are known to act in response to cellular stresses and are members of the MAPK family that
31 transduces signals through phosphorylation cascades [25, 26]. It also activates tyrosine kinase
32 receptors (RTKs), such as EGF and PDGF receptors [27, 28] and protein kinase C [29]. At
33 high concentrations, HNE is cytotoxic and shows its detrimental potential to cellular
34 compounds. Proteins are the primary targets for HNE, as it was reported that adducted
35 proteins are the second most abundant group of HNE-derivatives, just after HNE-GSH, HNA
36 and DHN metabolites. Proteins moderately modified with HNE are degraded in the
37 proteasome, whereas extensive modification leading to protein cross-links may result in the
38 formation of large aggregates that inhibit proteasome [30]. On the other hand, HNE may also
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3 directly inhibit proteasome, however, such effect was observed only at very high (1 mM and
4 higher) HNE concentrations [31]. High HNE concentrations also influence signaling
5 pathways, but usually the effects of its activity are different from those observed at
6 physiological doses. For example, at high concentrations HNE induces apoptosis through the
7 death receptor Fas, TP53 and liberation of cytochrome c from mitochondrial membranes [32].
8 Generally, HNE introduced into eukaryotic cells exhibits pleiotropic effects depending on the
9 concentration and the cell type.
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HNE also alters gene expression. In microarray experiments RKO cells treated with HNE responded by induction or downregulation of hundreds of genes. Among them, genes responsible for antioxidant response, heat shock and ER stress displayed the most significant changes in the quantity of transcripts. Nevertheless, transcription of genes whose products are engaged in many other processes, like cell signaling, apoptosis, cell cycle regulation or transcription regulation, was also influenced by HNE. This demonstrates that HNE simultaneously affects multiple cellular pathways, which in consequence gives rise to a complex response of the cell [32-34].

5. Modification of DNA bases by LPO products and their processing by DNA polymerases

Directly oxidized DNA bases, for years were considered major, harmful DNA lesions. It is now clear that modifications generated by LPO products may contribute to DNA damage to nearly the same extent as directly oxidized bases. There are distinct groups of linear and cyclic LPO specific DNA lesions.

Malondialdehyde reacts in DNA with guanine, adenine and cytosine to form cyclic pyrimido-[1,2 α]purine-10(3H)-one-2'-deoxyribose (M_1dG) adduct and linear N6-(3-oxopropenyl)-2'-deoxyadenosine (M_1dA) and N4-(3-oxopropenyl)-2'-deoxycytosine (M_1dC)

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3 adducts, respectively [9]. M₁dG (Fig. 6) is the major MDA-DNA adduct and was detected in
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5 human and rodent tissues. In *E. coli* cells, it induces transversions to T and transitions to A
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7 with a frequency comparable with that of 8-oxoG [4].
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10 Exocyclic propano DNA adducts are formed by the attachment of LPO product that generates
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12 additional saturated, six-membered ring in a DNA base. These adducts are derived from the
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14 reaction of DNA with acrolein, crotonaldehyde and HNE. Acr-dG, Cro-dG and HNE-dG were
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16 found in rodent and human tissues [35, 36].
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19 Etheno DNA adducts (Fig. 6) possess five-membered exocyclic rings attached to DNA
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21 bases. The exact pathway of their formation *in vivo* is not known, although it is clear that they
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23 may be generated *in vitro* by the exposition of DNA to lipid peroxides. Ethenoadducts are
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25 also introduced into DNA by certain environmental carcinogens like vinyl chloride or its
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27 metabolite, chloroacetaldehyde (CAA) [37].
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30 Propano- and etheno-type adducts that are found in native mammalian DNA are
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32 unsubstituted and substituted with side chains of different length, depending on the structure
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34 of reacting LPO product. For example, HNE may directly interact with guanosine, adenosine,
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36 cytidine and thymidine moiety in DNA. Michael addition, followed by Schiff base formation
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38 by HNE aldehyde group leads to formation of 1N²-propanodeoxyguanosine (Fig. 7) and
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40 plethora of HNE adducts to C, A and T. Thymine adducts are linear, due to the fact that
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42 thymine is deprived of exocyclic amino group. HNE and other LPO adducts to G, C and A are
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44 cyclic in nucleosides, nucleotides and single-stranded DNA. In double stranded DNA the
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46 reaction of aldehyde group is reversible. This results in the appearance of linear adducts,
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48 which are flexible and may be tolerated to some degree by DNA polymerases [38], on one
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50 hand, and on the other may form new Schiff base linkage with the amino- groups of nucleic
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52 acids and amino acids to create inter- and intra-strand DNA-DNA cross-links and DNA-
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54 protein crosslinks. In the presence of oxygen HNE undergoes epoxidation and reacts with G, C
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3 or A moieties in DNA that leads to ethenoadducts formation, substituted with heptyl side
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5 chains [10, 39] (Fig. 8).
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7 *In vitro* reaction of HNE with DNA bases is of different efficiency. dG and dC are
8 primary targets, while dA reacts with much lower efficiency, and HNE adducts to dT are in
9 minority [39]. Etheno-type adduct of HNE to guanosine, 1,*N*²-ε-guanosine, and propano-type
10 cyclic 1,*N*²-propanodeoxyguanine were shown to occur as four diastereoisomers of tetracyclic
11 structure (purine condensed with ethano and furano rings) [40, 41]. These isomers were shown
12 to have different mutagenic potency [42]. Other cyclic DNA adducts deriving from acrolein
13 and crotonaldehyde occur in stereomeric forms as well [43, 44].
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23 There is a lot of controversies concerning formation of unsubstituted etheno-DNA
24 adducts. It is suggested that they derive from HNE [45, 46]. In oxidizing conditions HNE may
25 form small amounts of unsubstituted etheno-DNA adducts, however the available data from
26 *in vitro* studies are not consistent. Chen and Chung [45] demonstrated that under oxidizing
27 conditions the ratio of unsubstituted to substituted ethenoadenine is 6:100, of unsubstituted to
28 substituted guanine equals 24:100, and the number of unsubstituted ethenoadducts is
29 significantly decreased under nitrogen. However, our previous *in vitro* studies showed that in
30 oxidizing conditions only guanosine yielded unsubstituted 1,*N*²-εdG, and only in the amount
31 of 1-2 % of modified dG [39]. Unsubstituted ethenoadducts are formed from unsaturated fatty
32 acids in animals and humans [47], however the exact pathway of their formation is not clear.
33 One suggested possibility of 1,*N*²-εdG formation is from HNE precursor, 4-hydroperoxy-2-
34 nonenal (HPNE) [48].
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52 **6. Sequence-specificity in the formation of exocyclic-DNA adducts and repair kinetics**

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54 Specific sequences in DNA, which favour formation of tertiary structures containing
55 single-stranded fragments, e.g. palindromic loops, cruciforms and GC-rich sequences may be
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1
2 particularly susceptible to formation of exocyclic DNA adducts. Studies of Chung and
3 coworkers [49] have shown that in human cells HNEdG adducts were formed preferentially in
4
5 the third guanine residue of the codon 249 of *TP53* gene. This site was found to be a
6
7 mutational hot-spot in numerous types of human cancers [50], which shows that tertiary DNA
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9 structure is of importance in the induction of DNA damage and/or its repair. It cannot be also
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11 excluded that LPO accompanies the development of different types of cancer, and that its
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13 fingerprint stays in DNA in the form of mutations in specific sequences.
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18 We also studied *in vitro* sequence-specific interaction of the vinyl -chloride metabolite
19 chloroacetaldehyde (CAA) with human *TP53* gene exons 5-8, using DNA Polymerase
20 Fingerprint Analysis (DPFA), and identified sites of the highest sensitivity for modification
21 using ethenoadducts-specific DNA glycosylases [51]. CAA-induced DNA damage was more
22 extensive in *TP53* regions which revealed secondary structure perturbations, and were
23 localized in regions of mutation hot-spots. These perturbations inhibited DNA synthesis on
24 undamaged template as well, suggesting that they may be the fragile sites for acquisition of
25 mutations due to the easier induction of lesions in these DNA sequences and/or substitution of
26 replicative DNA polymerases with DNA polymerases of lower fidelity, which incorporate
27 non-cognate nucleotides [52].
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40 Choudhury and coworkers [40] established that the half time of elimination of trans-4-
41 hydroxynonenal-induced cyclic 1,N²-propanodeoxyguanine DNA adducts by HeLa cells
42 nuclear extract was 3 h in 30°C. This repair was performed by nucleotide excision repair
43 system, as the extracts from XPA deficient cells did not eliminate the lesion from DNA.
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45 Interestingly, repair was stereospecific, two stereoisomers were removed from DNA more
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47 efficiently than the other two.
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56 7. Translesion synthesis of exocyclic-DNA adducts and their mutagenic potency

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3 The presence of unsubstituted etheno-adducted DNA bases in the template relatively
4 mildly inhibits DNA synthesis by replicative and damage-specific DNA polymerases, while
5 substituted etheno-type and propano-type cyclic adducts pose a strong barrier for replication
6 [53, 54].
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12 Ethenoadducts are mutagenic, however prokaryotic and eukaryotic DNA polymerases
13 may behave differently when replicating DNA containing ethenoadducts. In bacteria, 1,*N*⁶-
14 ethenoadenine is mostly recognized as unmodified adenine by DNA polymerases, and
15 infrequently (about 0.1%), even under SOS conditions (conditions of decreased replication
16 fidelity caused by production of the elevated amounts of damage specific DNA polymerases
17 of the Y family), gives rise to A:T → G:C transitions [55] or A:T → T:A and A:T → C:G
18 transversions, as well as to one-nucleotide deletions [56]. In contrast, in simian kidney (COS)
19 cells 70% of εA residues in DNA are replicated erroneously, giving rise to 63% of
20 A:T → G:C transitions and to only 7% of other base-pair substitutions [56]. Mutagenic
21 specificity of εA depends also on its position on leading and lagging strand during DNA
22 replication. In human HeLa cells A:T → T:A transversions were the most frequent (7%) on
23 the leading strand, although A:T → C:G and A:T → G:C base pair substitutions were also
24 numerous (5% and 2%, respectively) [57].
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42 Ethenocytosine in site-directed mutagenesis experiments in *E. coli* and mammalian
43 cells gave rise to 1.5 or 2% of targeted mutagenic events in non SOS-induced *E. coli* cells [55,
44 58], 30% in SOS-induced ones, and 81% in simian kidney cells [58, 59]. The predominant
45 mutations generated by εC in both *E. coli* and COS cells were C:G → A:T transversions and
46 C:G → T:A transitions.
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53 *N*²,3-ethenoguanine induced G:C → A:T transitions and 1,*N*²-ethenoguanine induced
54 G:C → A:T, G:C → T:A and G:C → C:G base-pair substitutions both in primer extension
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3 assays with purified DNA polymerases and in *E. coli* cells [60-62]. Additionally, 1,N²-εG
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5 generated *in vitro* one- and two-nucleotide deletions [63].
6

7 Treatment of mammals with compounds inducing etheno DNA adducts, additionally
8
9 triggers chromosomal aberrations and recombination [64]. It was shown that εA when present
10
11 in DNA strongly stimulates the activity of topoisomerase II, causing DNA strand breaks [65].
12

13
14 Substituted LPO-derived cyclic DNA adducts, like HNE-DNA adducts strongly inhibit
15
16 DNA synthesis by prokaryotic and eukaryotic DNA polymerases both *in vitro* and in cellular
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18 models [38, 39]. In bacteria HNE increased the mutation rate in the *lacZ* gene of M13 phage
19
20 transfected into wild type *E. coli* strain. The most frequent events were recombinations, base
21
22 substitutions and frameshifts. Over 50% of base substitutions were C → T transitions followed
23
24 by G → C and A → C transversions, which suggests that HNE adducts to all DNA bases are a
25
26 source of point mutations, as well as trigger recombination events [38, 39]. In *E. coli* HNE
27
28 induces the SOS system [66] with low fidelity DNA polymerases, Pol IV and Pol V, which
29
30 can bypass different DNA lesions, thus enabling the cell to survive under stress conditions.
31
32 The third SOS *E. coli* DNA polymerase, Pol II, is the enzyme of high fidelity. Detailed
33
34 analysis of the contribution of SOS DNA polymerases in the induction of mutations on HNE
35
36 damaged templates revealed that Pol IV can bypass HNE-DNA adducts in an error-free
37
38 manner. Pol II and Pol V are strongly blocked by the presence of these adducts. The
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40 infrequent bypass by Pol II is error-free, while by Pol V is error-prone. Both Pol II and Pol IV
41
42 can compete with Pol V decreasing mutation rate on HNE-adducted templates [67].
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48 Cooperation of low-fidelity DNA polymerases in bypassing HNE-DNA adducts was
49
50 also observed in eukaryotic cells. Pol ι was able to incorporate correctly matched nucleotide
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52 opposite the HNE-dG adduct, although it was not able to elongate the synthesized DNA
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54 strand and fell off the template. Pol κ, in turn, could elongate the HNE-dG:dC pair, which
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56 ensured further DNA synthesis [54]. In this way the HNE-dG adducts were bypassed by two
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3 damage-specific DNA polymerases in an error-free manner. This may, at least partially
4
5 explain the fact that HNE is the least mutagenic LPO product, however due to the fact that it
6
7 is also able to modify proteins, the most cytotoxic [20].
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10 11 12 **8. LPO-induced DNA damage in human pathological processes**

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14 Constantly growing body of evidence indicates that LPO products are engaged in
15
16 human pathology. Much of these data is devoted specifically to HNE and different aspects of
17
18 its reactivity. Measurements of protein adducts, DNA adducts and levels of free HNE in
19
20 human blood and tissues were performed. Presently there are nearly forty human diseases
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22 (neurodegenerative, hepatic, cardiovascular, metabolic and many others) in which increased
23
24 HNE steady state levels have been detected [20].
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30 31 **8.1. Exocyclic-DNA adducts, oxidative stress and cancer**

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33 The most abundant LPO-derived DNA adducts like propanoadducts to guanine and, to
34
35 a lesser extent, ethenoadducts, were found in many tissues of rodents and humans as
36
37 background DNA lesions [68]. However, there are also examples that in some conditions
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39 DNA ethenoadducts can be generated in the organism above the basic levels. Accumulation
40
41 of DNA ethenoadducts was reported in LEC rats, in which copper dependent induction of
42
43 oxidative stress *via* Fenton reaction occurs [69]. Similarly, an increase in ethenoadducts levels
44
45 was correlated with oxidative stress in OXYS rats overproducing ROS [70], and was reported
46
47 in spleen of SJL mice stimulated to overproduce nitric oxide (6-fold elevated for both ϵ A and
48
49 ϵ C lesions) [71]. The most recent paper on this issue reports that in SJL mice inflammation
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51 model, lipid peroxidation derived DNA lesions dominate over DNA oxidation and DNA
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53 deamination products. Importantly, DNA ethenoadducts (ϵ A and $1,N^2$ - ϵ G) were found to be
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55 elevated 3-4 fold not only in organs that were targeted by inflammatory processes, but also in
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3 kidneys, which are not directly affected by the inflammation. It was concluded that oxidative
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5 and nitrosative stresses associated with inflammation can affect tissues at a distance from the
6
7 activated macrophages responsible for NO overproduction during chronic inflammation [72].
8

9
10 Oxidative stress and LPO-derived DNA damage can play an important role in cancer
11
12 development, especially in cancers where inflammatory processes are a characteristic
13
14 component of their etiopathogenesis [73]. In the animal model positive correlation between
15
16 the level of ethenoadduct DNA lesions and lipoxygenase-catalyzed metabolites of arachidonic
17
18 acid was established in the study on multistage mouse skin carcinogenesis [74]. Since then,
19
20 numerous studies showed that in several human diseases predisposing to cancer, the levels of
21
22 ethenoadducts in cancer prone tissues were elevated (Table 1)
23

24
25 From the clinical perspective all these diseases are associated with chronic
26
27 inflammatory processes and high risk of cancer development. On the molecular level they are
28
29 considered as malfunctions that lead to oxidative stress in cells of the targeted organ.
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31
32 Currently it is postulated that persistent oxidative stress and lipid peroxidation
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34 induced by inflammation, metal storage diseases and unbalanced diet lead to accumulation of
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36 DNA lesions from endogenous sources. Under conditions of chronic and long term
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38 occurrence of these risk factors, perpetuation of carcinogenesis or progression of cancer to
39
40 full malignancy is possible [75, 76].
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45 **9. Repair of LPO derived DNA lesions**

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47 DNA is under permanent control of several defense systems, which have been
48
49 developed to protect the genome from mutagenic modifications. DNA repair systems are
50
51 specialized in repair of almost all possible damages, and the choice of the repair pathway
52
53 depends on the structure of the damage. Bulky adducts are repaired by Nucleotide Excision
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55 Repair pathway (NER) [77], double strand breaks and interstrand cross-link may be processed
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3 by Non Homologous End Joining (NHEJ) or Homologous Recombination (HR) [78], whereas
4
5 most of the small DNA lesions are repaired by the Base Excision Repair or by direct reversal
6
7 (alkyltransferases, dioxygenases) [79].
8

9
10 In repair of LPO-induced DNA damage probably all above-mentioned DNA repair
11
12 systems are engaged. This is due to a variety of the types of DNA lesions induced by LPO
13
14 products.
15

16 17 18 **9.1. Repair of etheno-DNA adducts by BER enzymes** 19

20
21 BER is a DNA repair pathway dedicated to processing of small base lesions derived
22
23 from oxidation and alkylation that cause only little distortion in the DNA helix. In a classical
24
25 view it consists of five steps: (I) lesion recognition, (II) excision of the damaged base, (III)
26
27 AP site incision and removal of the chemical residues that could block further steps of the
28
29 pathway, (IV) incorporation of the proper nucleotide(s) by DNA polymerases and (V) ligation
30
31 of the DNA strand to restore the original DNA molecule [80-83].
32

33
34 Damaged bases are recognized by DNA glycosylases. Glycosylases are specialized
35
36 enzymes capable of processing a wide range of oxidative and alkylated bases. Presently, 13
37
38 human DNA glycosylases with different, but partially overlapping substrate specificity have
39
40 been identified. Several are engaged in repair of etheno-DNA adducts. All of them are
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42 monofunctional DNA glycosylases, and excise damaged base leaving behind an abasic (AP)
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44 site. AP site is subsequently recognized by the next enzyme in the BER pathway, AP-
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46 endonuclease, which cleaves phosphodiester bond 5' to the AP site initiating further repair
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48 steps, deoxyribose excision, filling the gap and ligation.
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50
51 ϵ A is excised by alkylpurine-DNA-*N*-glycosylase (ANPG) [84], whereas ϵ C by
52
53 thymine-DNA-glycosylase (TDG); the latter also excises thymine from G:T pairs, resulting
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55 from deamination of 5-methylcytosine [85]. Ethenocytosine is also excised by single-stranded
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3 uracil DNA glycosylase, SMUG1 [86] and by MBD4 glycosylase, although activity of the
4
5 latter is strongly limited to CpG islands [87, 88].
6

7 ANPG glycosylase has a wide substrate specificity, and besides ϵ A excises from DNA
8
9 also 1, N^2 -ethenoguanine [89], hypoxanthine and the alkylated bases, 3-methyladenine and 7-
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11 methylguanine [84]. TDG excises ϵ C, T and U from pairs with guanine, and its activity is
12
13 strongly stimulated by the next enzyme in the BER pathway, AP-endonuclease, APE1 [90].
14
15 TDG and APE1 also play the role of transcription regulators [91-94]
16
17

18 Ethenocytosine repair by the BER system is dependent not only on the level and
19
20 activity of TDG, MBD4 or SMUG1 DNA glycosylases, but also on the presence and/or
21
22 activity of ANPG glycosylase, which primary substrate is ϵ A. ANPG binds strongly to DNA
23
24 containing ϵ C, and although does not excise this modified base, does not allow its excision by
25
26 the proper ϵ C glycosylase, like TDG [95]. This may explain observation that the level of ϵ C
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28 measured in different human tissues was constantly higher than that of ϵ A [96].
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34 **9.1.1. Repair of etheno-DNA adducts by BER system and human cancers**

35 We compared repair of ϵ A, ϵ C and 8-oxoguanine (8-oxoG) in polymorphic blood
36
37 leukocytes of non-small cell lung cancer (NSCLC) patients and healthy volunteers. The major
38
39 histological subtypes of NSCLC are squamous cell carcinoma (SQ), which ethiology is
40
41 strongly linked to tobacco smoking, and adenocarcinoma (AD), which is related to chronic
42
43 inflammations, infections and healing of scars, although, the majority of lung cancer patients
44
45 in Western countries are tobacco smokers or past smokers. The most striking finding of the
46
47 study was observation that blood leukocytes repair capacity for ϵ A and 8-oxoG was
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49 significantly lower in lung cancer patients than in healthy volunteers [97, 98]. Consistently, 8-
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51 oxoG level in cancer patients leukocytes was higher than that in healthy controls. This showed
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53 that deficient repair of oxidative DNA damage may be a risk factor for the development of
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3 lung cancer. Surprisingly, ϵ C repair rate was the same in the leukocytes of cancer patients and
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5 healthy controls. However, in individuals developing AD, ϵ C repair was significantly
6
7 decreased. Also, ϵ A repair activity was markedly lower in the leukocytes of AD patients than
8
9 in SQ patients and healthy controls. Comparing repair rates in lung tissues of cancer patients,
10
11 similar differences were observed between histological types of cancer. ϵ A and ϵ C-repair
12
13 activities were significantly lower in normal lung of individuals suffering from AD than from
14
15 SQ. Interestingly, no such differences were observed in repair rates of 8-oxoguanine. This
16
17 suggests that the etiology of lung adenocarcinoma may be related to inefficient repair of
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19 exocyclic DNA adducts, deriving from lipid peroxidation. Very few other studies have linked
20
21 LPO with the progression of lung cancer, and our study showed, for the first time, the
22
23 association between the deficiency of specific repair pathways for ϵ A and ϵ C with the
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25 development of specific histological type of tumor.
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30 No difference in ϵ A and ϵ C level between tumor and non-affected lung tissues was
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32 recorded, and this could be due to compensating increase of repair activities in the tumor. No
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34 significant differences in ϵ A and ϵ C level as well as repair activities were associated with age,
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36 gender and smoking habit.
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40 Thus results obtained in that study suggested that oxidative stress contributes to induction
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42 and/or progression of lung cancer, and that repair deficiency for ϵ A and ϵ C is associated
43
44 particularly with the development of lung adenocarcinoma.
45

46
47 Similarly, ϵ A and ϵ C repair rates were decreased in PBL of colorectal carcinoma
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49 (CRC) patients in comparison to healthy controls, suggesting that such decreased repair rate
50
51 could be one of the risk factors for CRC development [99]. Indeed, inflammation and high fat
52
53 diet are the major risk factors for CRC development. Surprisingly, ϵ A and 1, N^2 - ϵ G level was
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55 also lower in PBL of CRC patients than in healthy controls. This could suggest that apart
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57 from the BER system, ethenoadducts are eliminated from DNA by other repair activities,
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3 which are induced in cancer patients. Good candidates are dioxygenases of AlkB family.
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5 Their level was significantly increased in some types of cancer e.g. prostate cancer [100, 101]
6
7 however their contribution to repair of exocyclic DNA adducts in mammals still is not clear.
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10 11 **9.1.2. Oxidative dealkylation by AlkB enzymes**

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14 It was demonstrated that AlkB protein from *Escherichia coli*, which removes alkyl
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16 groups by direct reversal without DNA scission and resynthesis, repairs also ϵ A and ϵ C [102-
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18 104]. Cellular functions of numerous members of this group of enzymes is poorly elaborated,
19
20 and it is not well understood to what extent they participate in repair of exocyclic DNA
21
22 adducts. The data obtained for *E. coli* suggest that in this bacteria AlkB is at least as
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24 important as BER in the elimination of etheno-type, unsubstituted exocyclic DNA adducts
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26 [105].
27
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29
30 Dioxygenases from AlkB family remove alkyl groups from DNA by oxidative
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32 dealkylation in the reaction, which requires oxygen, 2-oxoglutarate and Fe^{2+} ions [2, 106]. *E.*
33
34 *coli* AlkB protein catalyses oxidation of cytotoxic alkyl groups in ssDNA, dsDNA as well as
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36 in RNA, which leads to the reconstitution of intact base and liberation of oxidized alkyl
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38 group, e.g. formaldehyde or glyoxal (Fig. 9) [2]. The main DNA lesions repaired by *E. coli*
39
40 AlkB protein are 1MeA and 3MeC, but the enzyme also repairs 1MeG, 3MeT, as well as
41
42 removes ethyl, propyl, hydroxypropyl groups and etheno-groups from exocyclic DNA
43
44 adducts, ϵ A and ϵ C [104, 107-109]. The enzyme removes also alkyl groups from mRNA and
45
46 tRNA [110].
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49
50 AlkB proteins were found in most organisms from RNA viruses to humans. *In silico*
51
52 search revealed the existence of nine AlkB homologues in human genome, ABH1-8 [111] and
53
54 the product of human obesity gene, *FTO* [112]. Biological functions of these proteins are not
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56 completely understood, however hABH1, hABH2, hABH3 and FTO were proven to have
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3 enzymatic activities dealkylating DNA or RNA [113]. ABH2 and ABH3 similarly to AlkB
4 dealkylate 1MeA, 3MeC, 1MeG, 1MeT and 1-EtA in DNA, and ABH3 repairs RNA as well
5 [106, 114, 115]. The best substrate for ABH3 is 3MeC and this lesion is preferentially
6 repaired in ssDNA [106, 116]. ABH2 eliminates more efficiently 1MeA than 3MeC, and
7 repair is more efficient when the lesion is localized in dsDNA than in ssDNA [114]. hABH2
8 eliminates also ϵ A from ds and ssDNA [117], and thus may be a backup enzyme for ANPG
9 glycosylase. Recently, it was shown that hABH2 repairs ϵ C, as well, and probably competes
10 with ANPG in the access to the lesion, since ANPG inhibits *in vitro* repair of ϵ C by ABH2
11 [118]. It is, however necessary to stress, that repair of etheno-DNA adducts by AlkB proteins
12 is much less efficient than repair of methylated bases. ABH2 was found in replication foci,
13 and contains a motif to bind PCNA [119]. The function of ABH2 might be to clean the
14 template or alternatively participate in replication regulation.

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ABH2 and ABH3 knockout mice are viable with phenotype similar to that of the wild
type animals. ABH2 knockout mice accumulate 1MeA with age [120]. However, no
accumulation of ϵ A was found both in ABH2 and ABH3 deficient mice. In contrast, a
significant increase of ϵ A level was observed in DNA of ANPG deficient mice [120]. This
suggests that in spite of the possibility to repair ϵ A *in vitro*, *in vivo* ABH2 is not able to cope
with endogenously formed ϵ A.

ABH1 is a mitochondrial protein and removes 3MeC from ssDNA and RNA [121].
Recent studies suggest that ABH1 may also possess a lyase activity [122].

FTO dealkylates 3MeT and 3MeU in ssDNA and RNA [123], as well as N^6 -MeA in
ssDNA and RNA [124]. It is suggested that in higher eukaryotes FTO may be a major
enzyme, which removes methyl group from N^6 -MeA in mRNA.

ABH8 besides 2-oxoglutarate/ Fe^{2+} -dependent dioxygenase domain contains also RNA
binding domain and methyltransferase domain. The protein is a tRNA methyltransferase

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3 required for the final step in the biogenesis of 5-methoxycarbonyl-methyluridine (mcm5(U)).
4
5 The interaction of ALKBH8 with a small accessory protein, TRM112, is required to form a
6
7 functional tRNA methyltransferase [125]. mcm5(U) is frequently found in the wobble position
8
9 of uridine, and may change the efficiency of translation of certain proteins [126]. Dioxygenase
10
11 activity of ABH8 participates in hydroxylation of mcm5(U) and its derivative 5-
12
13 methoxycarbonylmethyl-2-thiouridine (mcm5s2U) to yield 5-(S)-[methoxycarbonyl-
14
15 (hydroxy)methyl]uridine ((S)-mchm5U) [127].
16
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19 AlkB proteins may also play divergent roles not related to modification of nucleic
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21 acids. ABH1 homologue participates in differentiation of trophoblast stem cells and in the
22
23 epigenetic regulation of transcription [128]. ABH3 was recognized as prostate cancer antigen
24
25 1 (PCA-1), and many studies suggest that ABH3 may be a selective marker for malignant
26
27 transformation in prostate [100]. Recently, it was observed that PCA-1 increases expression of
28
29 Discoidin Domain Receptor 1, a receptor with tyrosine kinase activity, and in consequence
30
31 promotes prostate cancer development [129].
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35 The function of other human AlkB homologues is obscure. Their mRNA is quite
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37 abundant in most tissues, particularly in testis, which suggests that these proteins may play a
38
39 role in cell differentiation, division or other important functions, and that their role is not
40
41 limited to DNA repair [130].
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45 **9.1.3. Nucleotide excision repair and recombination**

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47 Bulky LPO adducts to DNA bases, like HNE adducts, which have alkyl side chains are
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49 repaired by nucleotide excision repair system (NER). Chung and coworkers [49, 131] showed
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51 that HNE-dG adducts are recognized and excised from DNA by bacterial UvrABC
52
53 excinuclease. HNE-dG adducts were also more cytotoxic and mutagenic in NER deficient
54
55 human and *E. coli* cells than in NER proficient cells [132]. Subsequent studies in *E.coli*,
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3 which used M13 phage model showed that HNE adducts to all DNA bases are efficiently
4 repaired by *E. coli* NER system, however in NER deficient strain a huge increase of
5 recombination events was observed [38], and mutation frequency increased significantly. This
6 suggested that both NER and recombination are engaged in the elimination of deleterious
7 effects of the presence of HNE-DNA adducts in genetic material. Interestingly, in *E. coli*
8 strains deficient in repair by NER and recombination the survival of M13 phage modified
9 with HNE was higher and mutation frequency lower than in the single *uvrA* mutant,
10 suggesting the presence of a backup repair system operating in the absence of NER and
11 recombination repair. The contribution of mismatch repair was excluded [38]. Next studies
12 [67] showed that SOS DNA polymerases may play a role of such a backup system, since Pol
13 IV and Pol II synthesized DNA faithfully on HNE damaged template in *E. coli* cells (see
14 paragraph 7).

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Contribution of recombination in repair of HNE-DNA adducts in mammalian cells
was reported already in the 90-ties, since HNE was shown to induce micronuclei, sister
chromatid exchanges and chromosomal aberrations [133-135].

Since most LPO-derived DNA adducts, like HNE-DNA adducts are able to form
interstrand DNA cross-links [136], other repair systems, namely Fanconi anemia pathway
may be engaged in repair of these DNA damages. This, however, needs further research.

We have also shown that the presence of bulky HNE-DNA adducts in the template
strongly inhibits transcription by T7 RNA polymerase, and HeLa cells extract *in vitro* [137].
In contrast, unsubstituted ϵ A did not inhibit RNA synthesis [138]. Treatment of wild type cells
with 1-20 μ M HNE caused dephosphorylation of the Cockayne syndrome complementation
group B (CSB) protein. Such dephosphorylation stimulates its ATPase activity of the CSB
protein necessary for transcription-coupled repair (TCR), a quick NER subpathway that
preferentially removes lesions from transcribed DNA strand. The deficiency of CSB protein

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3 leads to progressive multisystem degeneration and premature aging [139]. Cell lines
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5 expressing CSB protein mutated in different ATPase domains exhibited different sensitivities
6
7 to HNE. The motif II mutant, which binds ATP, but is defective in ATP hydrolysis was as
8
9 sensitive to HNE as CSB-null cells. In contrast, motif V mutant cells were as sensitive to
10
11 HNE as were the cells bearing wild type protein, while motif VI mutant cells showed
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13 intermediate sensitivity to HNE. These mutants exhibit decreased ATP binding, but retain
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15 residual ATPase activity. In addition, CSB-deficient cells were hypersensitive to
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17 physiological concentrations of HNE, and developed a higher level of sister chromatid
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19 exchanges in comparison to the wild type cells [137]. These results strongly suggest that
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21 HNE-DNA adducts are endogenous DNA lesions, blocking transcription in mammalian cells
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23 and are processed by the TCR system. Thus, HNE, and possibly other LPO products, might
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25 also be endogenously formed compounds contributing to accelerated aging and degeneration.
26
27 Indeed, increased level of HNE accumulation was observed in brain tissue and in cerebrum of
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29 patients suffering from several neurodegenerative disorders like Alzheimer's, Parkinson's,
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31 Pick's, amyotrophic lateral sclerosis and Huntington diseases [140]. Interestingly, large
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33 amounts of HNE-modified proteins were found in the brains (globus pallidus) of Cockayne
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35 syndrome patients [141].

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40 DNA repair proteins may also be targeted by LPO products, however, literature data
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42 on this issue are rather limited. Feng and coworkers [142] have shown that treatment of cells
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44 with HNE inhibits elimination of UV dimers and benzo[a]pyrenediol epoxide-guanine
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46 adducts from DNA, suggesting that NER may be inhibited in mammalian cells by elevated
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48 HNE concentrations. We have also demonstrated that high HNE concentrations (100-200 μM)
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50 inhibit *in vitro* ATPase activity of the CSB protein as well as the transcription machinery in
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52 HeLa cell-free extracts [137]. Inhibition of cellular repair capacity by LPO products may be
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54 an important, additional mechanism of pro-carcinogenic consequences of inflammation.
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10. Conclusions

Lipid peroxidation generates plethora of reactive aldehydes, which are relatively stable and bind to nucleic acids, proteins and cellular thiols. As far as mutagenic properties were shown for the majority of etheno- and propano-type adducts to DNA bases, the adverse effects of LPO on human health remains controversial. Etheno-DNA adducts were elevated in cancer-prone human diseases, and carcinogenic properties were described for chemical compounds introducing ethenoadducts to DNA bases, e.g. vinyl chloride. Adducts to DNA bases of longer LPO products, like HNE, very efficiently block transcription by prokaryotic and eukaryotic transcription machineries, and may favor cell senescence, and possibly aging of organisms and neurodegenerative processes. In addition, HNE binds to proteins, and may change cellular transcription and signaling and this may depend on compound concentration. At high concentrations HNE inhibits repair of bulky adducts, which may be introduced to DNA by UV light, several environmental carcinogens, like benzo[a]pyrene, as well as LPO products, this way sensitizing cells to other mutagenic and carcinogenic exposures. On the other hand, increased HNE levels in cells also promote apoptotic signaling, while at decreased concentration, below its basal constituted levels, HNE promotes proliferation. It was shown that by blocking detoxification of HNE through the inhibition of RLIP76 catalyzed transport of glutathione-HNE out of the cell, a complete remission of many human cancer xenografts in mice was achieved [143]. HNE was also shown to inhibit telomerase activity in colon carcinoma cell lines [144] and in human leukemic cells [145]. This occurred by the reduction of GSH content, and by the modulation of expression and activity of transcription factors belonging to the Myc/Mad/Max network. Since the presence of PUFAs in the diet exposes epithelial colon cells to HNE, this aldehyde could contribute to cell growth control through the inhibitory action on telomerase activity and its expression, suggesting a protective effect

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3 on colon mucosa. Understanding of these processes in future may contribute to the
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5 improvement of cancer therapy.
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Table 1. DNA ethenoadducts levels in various human diseases.

Disease	Affected organ	Absolute values of ethenoadducts per 10 ⁹ parent nucleotides	Fold increase of DNA ethenoadducts	Reference
Wilson's disease	Liver	$\epsilon\text{A} - 61.03 \pm 7.95$ $\epsilon\text{C} - 91.50 \pm 36.02$	3 ϵA , 3 ϵC in comparison to normal liver	[71]
Primary hemochromatosis	Liver	$\epsilon\text{A} - 46.62 \pm 32.83$ $\epsilon\text{C} - 64.32 \pm 11.55$	2 ϵA , 2 ϵC in comparison to normal liver	[71]
Familial adenomatous polyposis	Colon polyps	$\epsilon\text{A} - 65.3$ $\epsilon\text{C} - 31.4$	2 ϵA , 2 ϵC in comparison to unaffected colon	[146]
Crohn's disease	Colon	$\epsilon\text{C} - 325 \pm 346$	20 ϵC in comparison to asymptomatic colon	[147]
Ulcerative colitis	Colon	$\epsilon\text{C} - 69 \pm 36$	4 ϵC in comparison to asymptomatic colon	[147]
Chronic pancreatitis	Pancreas	$\epsilon\text{A} - 63 \pm 56$ $\epsilon\text{C} - 354 \pm 251$	3 ϵA , 28 ϵC in comparison to normal pancreas	[147]

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3 **Figure 1. Pathways of lipid peroxidation.**

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5 **Figure 2. Structures of LPO-derived reactive aldehydes** (according to [7]). Left panel-
6 general structures, right panel- examples of reactive aldehydes that belong to particular
7 families, HHE- 4-hydroxy-2-hexenal, HNE- 4-hydroxy-2-nonenal, MDA- malondialdehyde,
8 ONE- 4-oxo-2-nonenal.

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10 **Figure 3. Mechanisms of HNE formation** (according to [17]). **A-** from 9-hydroxyperoxy-
11 octadecenoic acid (9(S)-HPODE) as a precursor; **B-** from 13-hydroxyperoxy-octadecenoic
12 acid (13(S)-HPODE) as a precursor.

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14 **Figure 4. Michael addition of thiols** (according to [10]).

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16 **Figure 5. Schiff-base formation with primary amines** (according to [10]).

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18 **Figure 6. The major exocyclic DNA adducts.** M₁dG, 1,N²-ethenoguanine (1,N²-εG), N²,3-
19 ethenoguanine (N²,3εG), 3,N⁴-ethenocytosine (εC), 1,N⁶-ethenoadenine (εA).

20
21 **Figure 7. Interaction of HNE with deoxyguanosine.**

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23 **Figure 8. Interaction of HNE epoxide with deoxyguanosine.**

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25 **Figure 9. AlkB mechanism of direct reversal.**

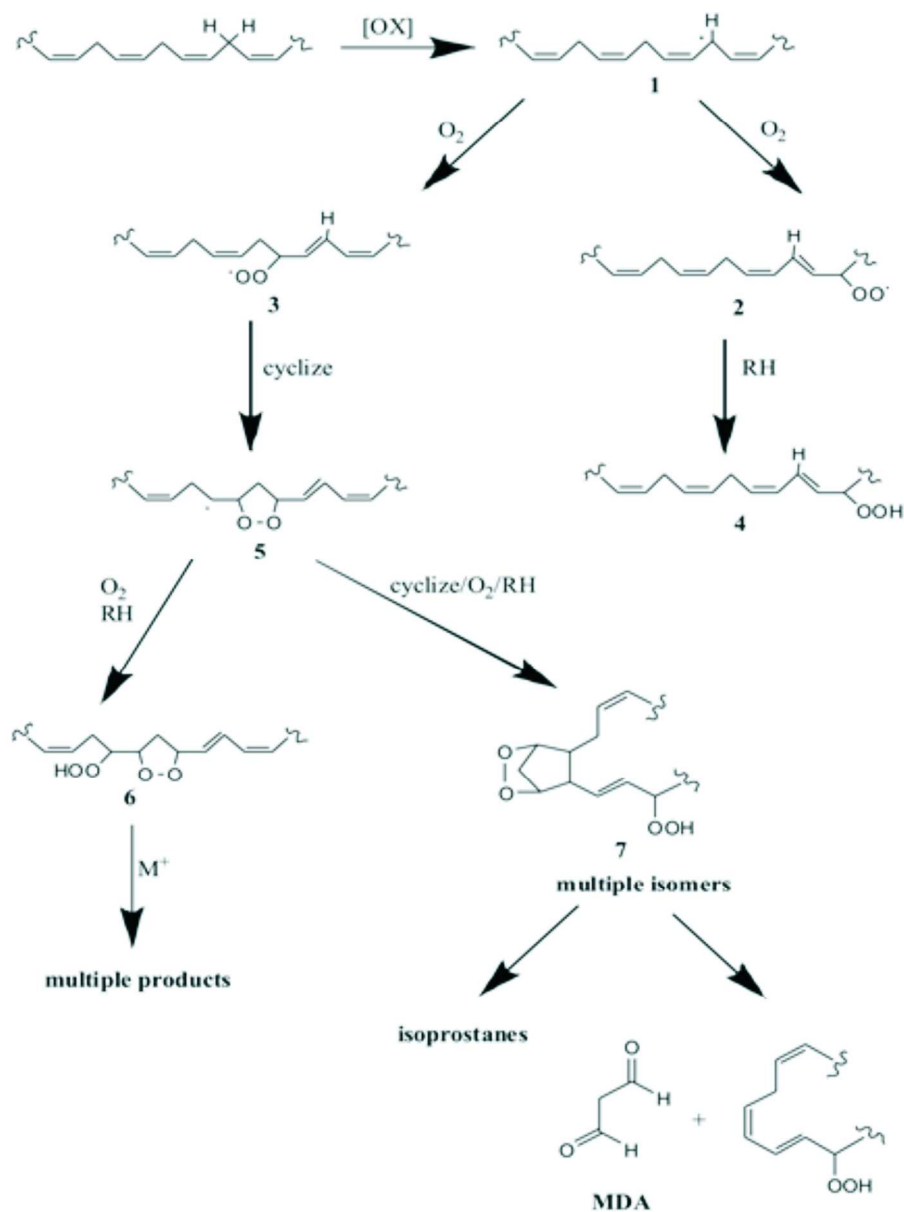


Figure 1. Pathways of lipid peroxidation
153x204mm (300 x 300 DPI)

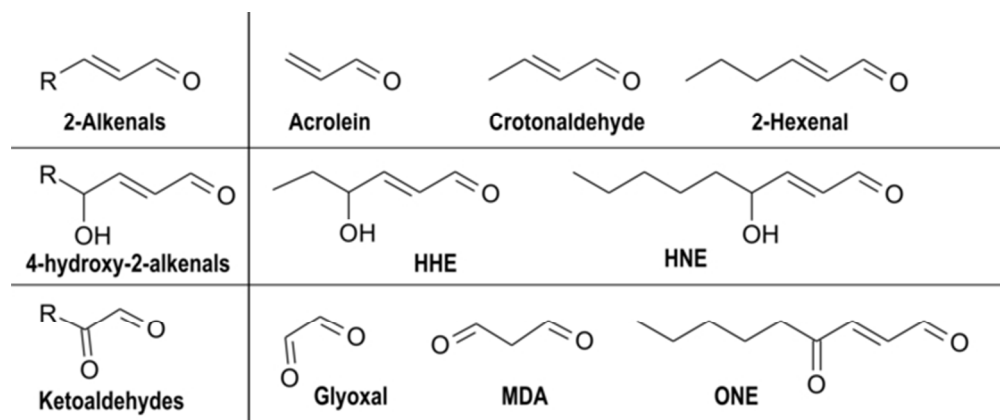


Figure 2. Structures of LPO-derived reactive aldehydes (according to [7]). Left panel- general structures, right panel- examples of reactive aldehydes that belong to particular families, HHE- 4-hydroxy-2-hexenal, HNE- 4-hydroxy-2-nonenal, MDA- malondialdehyde, ONE- 4-oxo-2-nonenal

59x24mm (300 x 300 DPI)

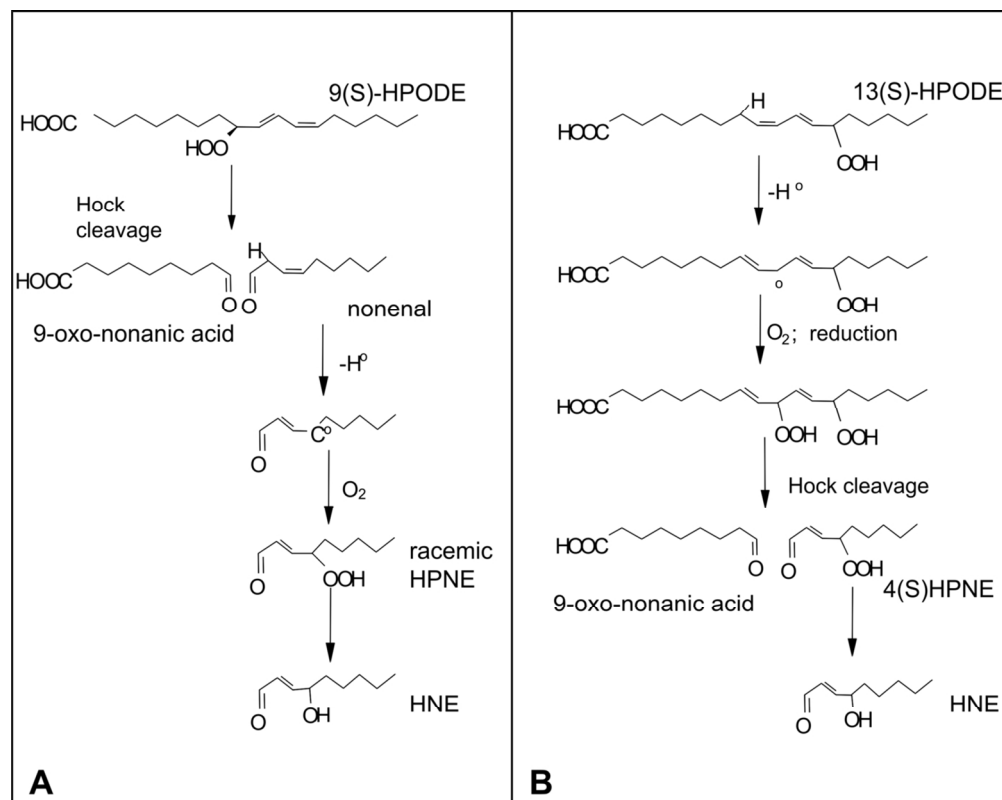
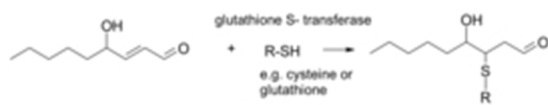


Figure 3. Mechanisms of HNE formation (according to [11]). A- from 9-hydroxyperoxy-octadecenoic acid (9(S)-HPODE) as a precursor; B- from 13-hydroxyperoxy-octadecenoic acid (13(S)-HPODE) as a precursor.
117x93mm (300 x 300 DPI)



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Figure 4. Michael addition of HNE to thiols (according to [10])
23x4mm (300 x 300 DPI)

For Peer-Review Only

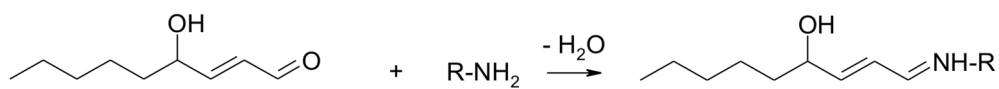
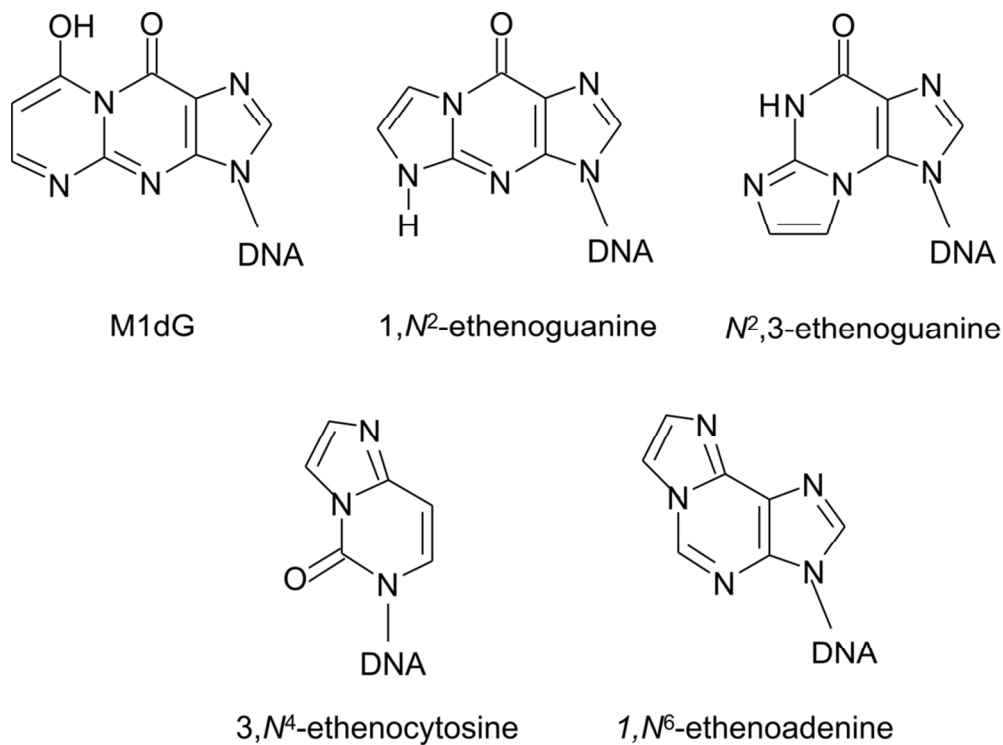


Figure 5. Schiff-base formation with primary amines (according to [10])
132x11mm (300 x 300 DPI)



32 Figure 6. Major modifications of DNA bases by LPO products. M1dG, 1,*N*²-ethenoguanine (1,*N*²-εG), *N*²,3-
33 ethenoguanine (*N*²,3-εG), 3, *N*⁴-ethenocytosine (εC), 1,*N*⁶-ethenoadenine (εA)
34 99x73mm (300 x 300 DPI)

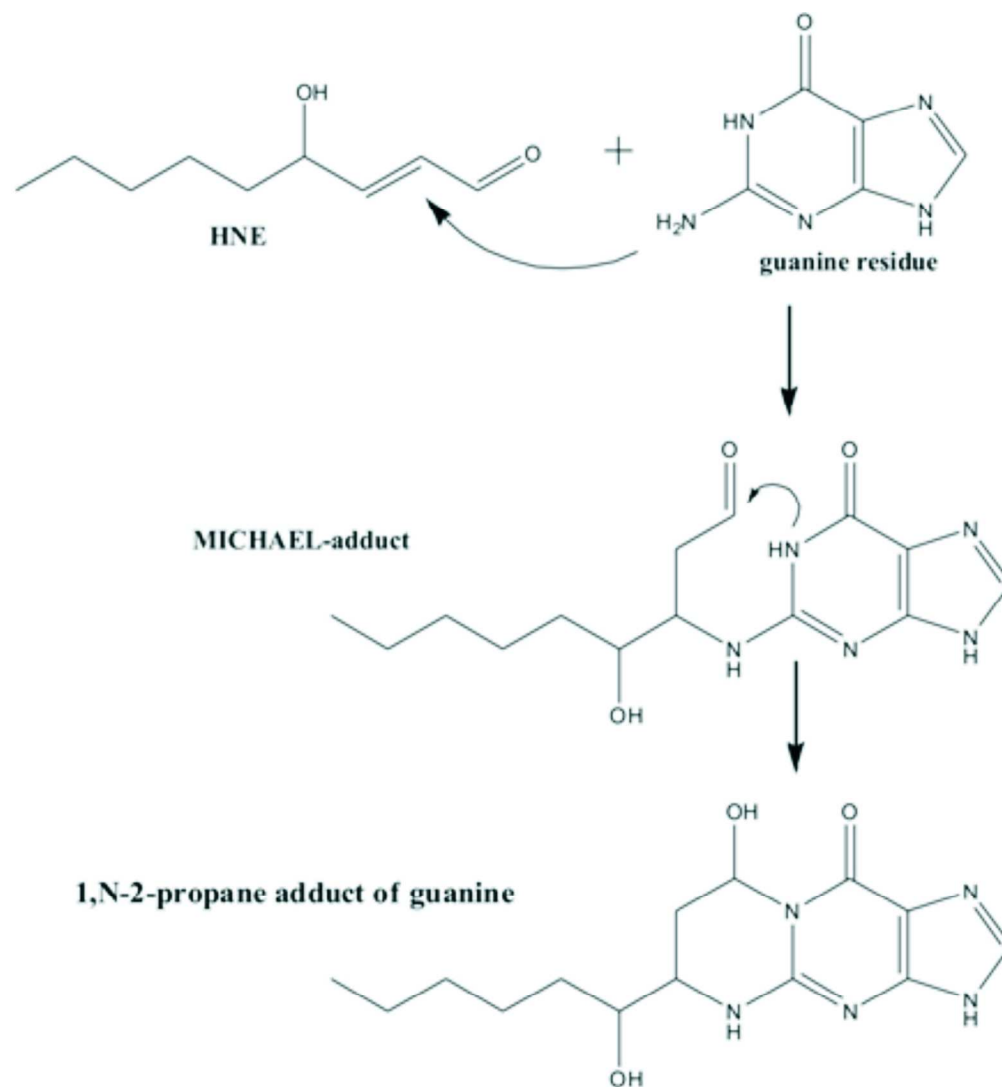


Figure 7. Interaction of HNE with deoxyguanosine
123x133mm (300 x 300 DPI)



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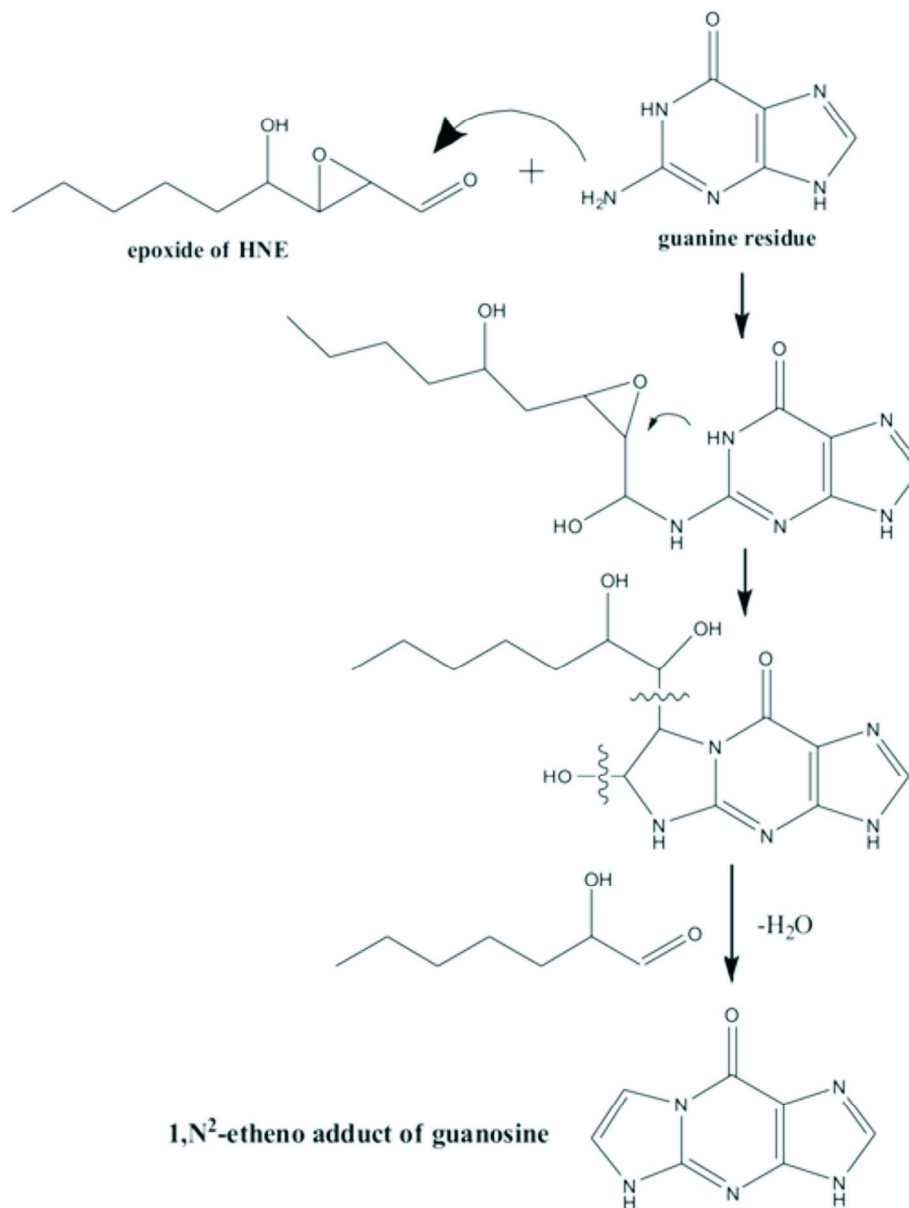
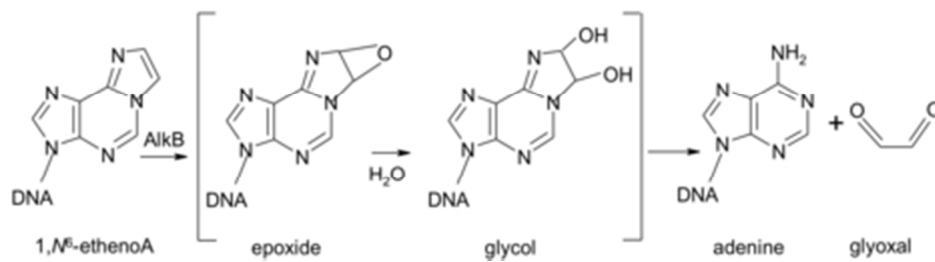


Figure 8. Interaction of HNE epoxide with deoxyguanosine
173x227mm (300 x 300 DPI)



16 Figure 9. The mechanism of oxidative dealkylation of DNA bases by AlkB proteins
17 39x10mm (300 x 300 DPI)

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