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Lipid peroxidation product 4-hydroxy-2-nonenal modulates base excision repair in human cells

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ABSTRACT

Oxidative-stress-driven lipid peroxidation (LPO) is involved in the pathogenesis of several human diseases, including cancer. LPO products react with cellular proteins changing their properties, and with DNA bases to form mutagenic etheno-DNA adducts, removed from DNA mainly by the base excision repair (BER) pathway.

One of the major reactive aldehydes generated by LPO is 4-hydroxy-2-nonenal (HNE). We investigated the effect of HNE on BER enzymes in human cells and *in vitro*. K21 cells pretreated with physiological HNE concentrations were more sensitive to oxidative and alkylating agents, H_2O_2 and MMS, than were untreated cells. Detailed examination of the effects of HNE on particular stages of BER in K21 cells revealed that HNE decreases the rate of excision of 1,N^6-ethenoadenine (εA) and 3,N^4-ethenocytosine (εC), but not of 8-oxoguanine. Simultaneously HNE increased the rate of AP-site incision and blocked the religation step after the gap-filling by DNA polymerases. This suggested that HNE increases the number of unrepaired single-strand breaks (SSBs) in cells treated with oxidizing or methylating agents. Indeed, preincubation of cells with HNE and their subsequent treatment with H_2O_2 or MMS increased the number of nuclear poly(ADP-ribose) foci, known to appear in cells in response to SSBs. However, when purified BER enzymes were exposed to HNE, only ANPG and TDG glycosylases excising εA and εC from DNA were inhibited, and only at high HNE concentrations. APE1 endonuclease and 8-oxoG-DNA glycosylase 1 (OGG1) were not inhibited. These results indicate that LPO products exert their promutagenic action not only by forming DNA adducts, but in part also by compromising the BER pathway.

Abbreviations: Acr, acrolein; ANPG, alkyl-*N*-purine glycosylase; AP-site, apurinic/apyrimidinic site; APE1, AP-site endonuclease; BER, base excision repair; BSA, bovine serum albumin; CAA, choloroacetaldehyde; CRC, colorectal cancer; Cro, croton aldehyde; CSB, Cockayne syndrome group B; DTT, dithiothreitol; εA, 1N^6-ethenoadenine; εC, 3N^4-ethenocytosine; 8-oxoG, 8-oxoguanine; EDTA, ethylenediaminetetraacetic acid; EH, 2,3-epoxy-4-hydroxynonanal; HHE, 4-hydroxy-2-hexenal; HNE, *trans*-4-hydroxy-2-nonenal; Fpg, formamidopyrimidine DNA glycosylase (functional homolog of human OGG1); GST, glutathione *S*-transferase; IPTG, isopropyl β-D-1-thiogalactopyranoside; LPO, lipid peroxidation; MBD4, methyl-CpG-binding domain protein 4 (DNA glycosylase specific for CpG sites); MDA, malondialdehyde; MMS, methyl methanesulfonate; MSI, microsatellite instability; OGG1, 8-oxoguanine glycosylase 1; PAR, poly(ADP-ribose); PBS, phosphate buffered saline; PNK, polynucleotide kinase; PUFA, polyunsaturated fatty acids; SSBs, DNA single strand breaks; TCR, transcription-coupled repair; TDG, thymine DNA glycosylase; hTERT, human telomerase reverse transcriptase; THF, tetrahydrofurane (analog of AP site); XRCC1, X-ray repair cross-complementing protein 1.

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1. Introduction

A growing body of evidence suggests that many of the detrimental cellular effects observed under oxidative stress conditions may be mediated by products of lipid degradation [1]. Polyunsaturated fatty acids (PUFA) are particularly susceptible to peroxidation, giving rise to 2-alkenals (e.g., acrolein (Acr), crotonaldehyde (Cro) 2-hexenal), 4-hydroxy-2-alkenals (e.g., 4-hydroxy-2-hexenal (HHE), 4-hydroxy-2-nonenal (HNE)), and ketoaldehydes (e.g., malondialdehyde (MDA), glyoxal, 4-oxo-2-nonenal (ONE)) [1,2]. These products are relatively stable and can diffuse throughout the whole cell attacking other biomolecules. The modification of nucleic acids and proteins by LPO products can have serious adverse effects on the cellular metabolism.

Modification of DNA bases by LPO products is a subject of extensive research, and distinct groups of linear and cyclic LPO DNA base adducts have been identified. Among them are etheno DNA adducts [3,4], which have a five-membered exocyclic ring attached to a DNA base. The exact pathway of their formation *in vivo* is unclear, but it is accepted that they may be generated by the exposure of DNA to lipid peroxides or to certain environmental carcinogens like vinyl chloride or its metabolite, chloroacetaldehyde (CAA) [5]. Ethenoadducts to adenine and cytosine as well as Acr-dG, Cro-dG and HNE-dG adducts have also been found in unexposed rodent and human tissues, suggesting their endogenous formation [6–8].

Most of the exocyclic DNA adducts have a strong mutagenic potential and many have been associated with human pathologies. Ethenoadducts have been implicated in the etiology of human cancer-promoting diseases. The levels of 1,N^6-ethenoadenine (εA) and 3,N^4-ethenocytosine (εC) are increased in Wilson disease [9], hemochromatosis [9], familial adenomatous polyposis [10], and Crohn's disease [11]. It has also been reported that adducts of 4-hydroxy-2-alkenals to DNA bases, but also to proteins, could be engaged in aging and neurodegeneration [12,13].

4-Hydroxy-2-nonenal (HNE) adducts to DNA bases inhibit DNA replication by prokaryotic and eukaryotic DNA polymerases [14,15]. HNE–DNA adducts in the template also inhibited transcription *in vitro*, both by T7 RNA polymerase and by a HeLa cell-free extract [15], implying that *in vivo* these lesions could be preferentially removed from the transcribed DNA strand by the transcription-coupled repair (TCR) system.

LPO products can also form adducts to proteins, but much less is known about the nature and biological consequences of such LPO-protein adducts than on their DNA counterparts. HNE is an amphiphilic compound with three functional chemical groups which determine its reactivity. The interplay of a double CC bond, a carbonyl group (CO) and a hydroxyl group (OH) produces partial positive charges at carbons 3 and 1, subject to nucleophilic attack by other compounds (e.g., thiols or amino groups). Thus, HNE may undergo Michael addition with biomolecules containing amino and/or thiol groups like cysteine, lysine, or the imidazole group of histidine [2]. HNE can also form Schiff bases with primary amines (e.g., lysine). This reaction is competitive with Michael addition [2]. Within cells HNE contributes to cross-linking of proteins by the reaction of the HNE aldehyde group with a lysine of a protein (Schiff base formation) and subsequent Michael addition to a cysteine or lysine of another protein [2].

The most reactive toward HNE is Cys, which undergoes HNE addition two and three orders of magnitude more efficiently than do His and Lys, respectively [16]. However, the actual site of modification of proteins by HNE is determined not only by the reactivity, but also by additional factors like local polarity, tertiary structure of the protein or accessibility of the side chain to HNE. There is ample data documenting protein modification by HNE *in vitro* and *in vivo*. HNE targets a variety of oxidoreductases, transferases, hydrolases, lyases, kinases, ion channels and many other proteins. In general, HNE adducts to proteins diminish their activity, although some examples of activation are also known [16].

One of the HNE effects is inhibition of some DNA repair enzymes. HNE inhibits the excision of UV-induced pyrimidine dimers and benzo[*a*]pyrene-guanine adducts by Nucleotide Excision Repair in HCT116 and A549 cells [17]. *In vitro*, high concentrations of HNE (50–200 μM) also inhibit the ATPase activity of the CSB protein, which may result in inhibition of the TCR pathway [15]. The above data, notwithstanding the effects of protein modifications by HNE and other LPO products on DNA repair processes, are by far less thoroughly understood than those of corresponding DNA lesions.

Here we address the question whether and to what extent HNE affects the Base Excision Repair pathway. BER usually removes small DNA-base modifications, like oxidatively derived base lesions, AP sites and alkylated bases, among them etheno-DNA adducts. In the classical view BER consists of five steps: (I) lesion recognition, (II) excision of the damaged base, (III) AP site incision and removal of chemical residues that could block further steps of the pathway, (IV) incorporation of the proper nucleotide(s) by DNA polymerases, and (V) ligation of the repaired DNA strand to restore the original DNA molecule [18–21]. 8-Oxoguanine (8-oxoG) is excised from DNA mostly by OGG1 glycosylase [22], 1,N^6-ethenoadenine (εA) by alkylpurine DNA *N*-glycosylase (ANPG) [23], whereas 3,N^4-ethenocytosine (εC) by thymine DNA glycosylase (TDG) [24]; the latter one also excises thymine from dG:dT pairs resulting from deamination of 5-methylcytosine [25]. ANPG and TDG are monofunctional DNA glycosylases and require an AP endonuclease to incise DNA at the site of the removed base to continue the BER process. 3,N^4-ethenocytosine is also excised by MBD4 glycosylase although its activity is strictly limited to CpG islands [26,27].

ANPG glycosylase has a wide substrate specificity and besides εA excises from DNA also 1,N^2-ethenoguanine [28], hypoxanthine [29] and the alkylated bases 3-methyladenine and 7-methylguanine [30]. TDG excises εC, thymine and uracil from pairs with guanine, and its activity is strongly stimulated by the next enzyme in the BER pathway, the AP endonuclease APE1 [31]. TDG and APE1 also act as transcription regulators [32–34].

Here we show that HNE affects several steps of the BER pathway, by causing an imbalance between the consecutive stages of the pathway. This leads to sensitization of cells to other genotoxins by increasing the level of DNA strand breaks due to unfinished repair.

2. Materials and methods

2.1. Cell lines

Human fibroblast cell line K21 immortalized with hTERT was a kind gift from Prof. M.H.K. Linskens, University of Groningen, The Netherlands. Cells were cultured in F10 Medium (+L-glutamine, Gibco), supplemented with 10% foetal bovine serum (Gibco) and grown at 37 °C in 5% CO_2 in 100-mm cell culture dishes.

2.2. Cell viability after HNE treatment

Cell viability was estimated by the trypan blue method. At approximately 90% confluency (3–4 mln cells per dish), cells were treated with different doses of HNE (0, 50, 100, 150 μM) for 2 h. HNE was then removed, cells were washed with 1× PBS and scraped from the plates, resuspended in F10 medium and 30 μl of the suspension was mixed with an equal volume of trypan blue dye (Sigma). Subsequently cells were counted under the microscope in a Neubauer chamber and the ratio of navy blue (dead) cells to