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Review Article

Genotoxicity of lipid oxidation compounds

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ABSTRACT

Lipid peroxidation, the oxidative degradation of membrane lipids by reactive oxygen species generates a large variety of breakdown products such as alkanes, aldehydes, ketones, alcohols, furans and others. Due to their reactivity aldehydes (alkanals, 2-alkenals, 2,4-alkadienals, 4-hydroxyalkenals) received a lot of attention, in particular because they can diffuse from the site of formation and interact with proteins and nucleic acids thus acting as second toxic messengers. The major aldehydic peroxidation product of membrane lipids is 4-hydroxynonenal (HNE). Since HNE and other 4-hydroxyalkenals are strong alkylating agents they have therefore been considered to be the biologically most important peroxidation products.

Although initially research focused on the toxicological potential of these compounds it is now well known that they play also a crucial role in cell signaling under physiological and pathophysiological conditions.

Thus, it is obvious that the biological effects will be determined by the intracellular concentrations which can trigger adaptation, DNA damage and cell death. This review will not cover all these aspects but will concentrate on the genotoxic properties of selected lipid oxidation products important in the context of pathophysiological developments together with a chapter on epigenetic modifications.

1. Introduction

Lipid peroxidation, the oxidative degradation of membrane lipids by reactive oxygen species generates a large variety of breakdown products such as alkanes, aldehydes, ketones, alcohols, furans and others [1–4]. Due to their reactivity aldehydes (alkanals, 2-alkenals, 2,4-alkadienals, 4-hydroxyalkenals) received a lot of attention, in particular because they can diffuse from the site of formation and interact with proteins and nucleic acids thus acting as second toxic messengers [5]. The major aldehydic peroxidation product of membrane lipids is 4-hydroxynonenal (HNE) [6]. Since HNE and other 4-hydroxyalkenals are strong alkylating agents they have therefore been considered to be the biologically most important peroxidation products [5].

Although initially research focused on the toxicological potential of these compounds it is now well known that they play also a crucial role in cell signaling under physiological and pathophysiological conditions [7–9]. Thus, it is obvious that the biological effects will be determined by the intracellular concentrations which can trigger adaptation, DNA damage and cell death. This review will not cover all these aspects but will concentrate on the genotoxic properties of selected lipid oxidation products (Fig. 1) important in the context of pathophysiological developments together with a chapter on epigenetic modifications.

2. Malonaldehyde (MDA)

Since it is generated from most fatty acids with more than two double bonds [10], MDA quantitatively is the major product of lipid peroxidation, i.e. HNE is formed in up to 80-fold lower concentrations [11]. Mutagenicity of MDA to Salmonella typhimurium was first demonstrated in 1976 by Mukai and Goldstein [12,13]. However, approximately 50% of the activity could later be attributed to impurities with higher mutagenic activity [14], but the application of highly purified MDA confirmed the mutagenic potential [15,16]. By reacting MDA at neutral pH with a single-stranded M13 vector containing the *lacZα* gene and analysis of mutations in this gene after replication in *E. coli* the following mutations were found: 76% of the mutations were base pair substitutions, of which 43% were transversions mostly of the G→T type. Transitions accounted for 53% and comprised exclusively C→T and A→G. Frameshift mutations occurred in 16% of the mutations [17]. In a different investigation by transfecting human fibroblasts with a shuttle vector, which had been allowed to react with MDA, and sequence analysis of the reporter gene after replication, Niedernhofer et al. [18] observed that the majority of mutations occurred at GC base pairs and that the most frequent mutations were large insertions and deletions and base pair substitutions. MDA has further been shown to induce genotoxicity in mammalian cells, i.e. methotrexate resistant mutations in the mouse lymphoma assay at concentrations ≥20 μM

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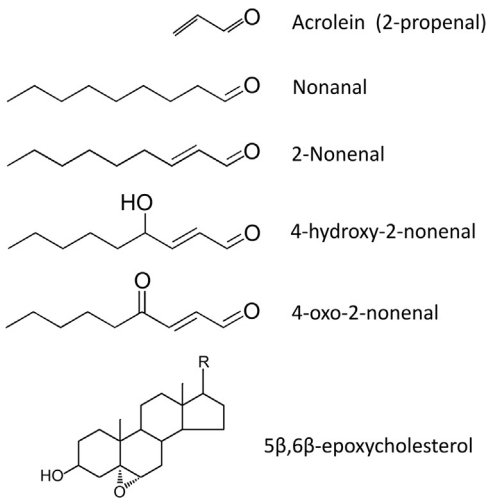

Fig. 1. Examples of molecular structures of the lipid oxidation products treated in this review.

[19], chromosomal aberrations and micronuclei in rat skin fibroblasts at concentrations ≥100 μM [20] and both single strand breaks and sister chromatid exchanges in CHO cells [21].

The mutational spectrum of MDA is related to its reaction with DNA at physiological pH to form guanosine, adenosine and cytidine adducts [22–26]. When reacting with guanosine both carbonyls react with nitrogen (N^2 and N^1) to form pyrimido[1,2 α]purin-10(3*H*)-one (M_1G), which is the most abundant MDA adduct. Adenosine and cytidine adducts arise from addition of one carbonyl to the exocyclic amino groups to form N^6-(3-oxopropenyl)deoxyadenosine (M_1A) and N^4-(3-oxopropenyl)deoxycytidine (M_1C), respectively. While M_1C is formed only in trace amounts, M_1A yields ~20% of M_1G [27]. By applying ^{32}P postlabeling Vaca et al. [28] were able to demonstrate that M_1G adducts are detectable in tissues from healthy humans. The levels were found to be 2.6 ± 1.2 adducts per 10^7 nucleotides in white blood cells and 3.0 ± 1.3 per 10^7 nucleotides in breast tissue. It has further been shown that the adduct level can be influenced by the diet. Healthy individuals of both sexes and different age received either a sunflower oil-based diet rich in polyunsaturated fatty acids or a rapeseed oil-based diet rich in monounsaturated fatty acids. The result was a 3.6 fold higher adduct level in the sunflower oil-based diet group [29]. Applying gas chromatography/mass spectrometry with electron capture negative chemical ionization detection it was further shown that the level of M_1G is in the range of 0.5–0.8 per 10^7 nucleotides in white blood cells [30] in the range of 5–12 per 10^7 nucleotides in liver [31] and in the range of < 0.1–5 in the pancreas [32].

MDA has also been shown to react with exocyclic amines on guanine residues to form interstrand crosslinks [18]. Such lesions are critical if not repaired because of blocking replication. MDA also readily forms crosslinks between DNA and histones under physiological conditions [33]. These are relatively stable at 37 °C with $t_{1/2}$ = 13.4 days. The authors further showed that crosslinking of histones to DNA proceeds through the initial formation of a protein adduct followed by reaction with DNA. Modification of DNA by malondialdehyde does not lead to a subsequent crosslinking of proteins.

3. Alkanals

The mutagenicity of 5*n*-alkanals was tested in V79 Chinese hamster lung cells [34] either at the hypoxanthine-guanine phosphoribosyltransferase locus as resistance to 6-thioguanine or at the Na/K ATPase locus as resistance to ouabain and resulted in a dose-dependent increase in the frequency of resistant mutants at concentrations ranging from 3 to 30 mM for propanal, butanal, pentanal and hexanal. Nonanal proved to be more genotoxic inducing mutations at concentrations of 0.1–0.3 mM. The same alkanals were also tested in primary cultures of rat and human hepatocytes based on the induction of unscheduled DNA synthesis (UDS) [35]. While there was a modest but significant increase of UDS for propanal, butanal, pentanal and hexanal in rat but not human hepatocytes, nonanal failed to induce UDS in both cell types although it showed the highest cytotoxicity. The authors therefore concluded that *n*-alkanals are presumably unable to induce genotoxic effects in the human liver.

4. 2-Alkenals

2-Hexenal, 2-heptenal, 2-octenal and 2-nonenal mutagenicity was tested in bacterial test systems [16] and caused effects in the micromolar range. The same 2-alkenals and 2-pentenal were tested in V79 Chinese hamster cells at concentrations ranging from 0.003 to 0.3 mM. All 5 2-alkenals induced a dose-dependent increase in the frequency of 6-thioguanine resistant mutants, and their mutagenic potency was found to increase with the length of the carbon chain [36]. In primary rat hepatocytes 2-nonenal induced significant levels of sister chromatid exchanges (SCE) at concentrations of 0.1 and 10 μM but no significant levels of micronuclei or chromosomal aberrations [37].

Being naturally present in various fruits and foods [38] hexenal received particular attention in this group. In human blood lymphocytes and cells of the permanent Namalva line hexenal was tested together with crotonaldehyde and nonadienal at concentrations of 5–250 μM or 5–70 μM (nonadienal). The endpoints analyzed were sister chromatid exchanges (SCE), chromosomal aberrations and micronucleus formation [39]. All 3 compounds induced dose-dependent increases of SCE and micronuclei, while structural chromosomal aberrations were significantly increased only by crotonaldehyde. In contrast, hexenal and nonadienal were potent inducers of aneuploidy while crotonaldehyde did not induce numerical chromosome aberrations. The authors therefore concluded that crotonaldehyde acts more as a clastogen whereas hexenal and nonadienal preferentially induce aneugenic effects. The same research group also conducted a study with healthy volunteers [40]. After rinsing the mouth with an aqueous 10 ppm solution of 2-*trans*-hexenal during 3 consecutive days micronucleus formation was determined in exfoliated buccal mucosa cells and revealed a doubling of the micronucleus frequency. In an additional study volunteers were examined before and after eating 3–6 bananas – containing approximately 35 ppm of hexenal – per day over a period of 3 days. Six of the 7 individuals showed at least a doubling of the micronucleus frequency [40].

The Comet FISH technique [41] further revealed that the KRAS oncogene and the tumor suppressor genes APC and TP53 were contained in the tails of primary human colon cells and colon adenoma cells (LT97) after treatment with hexenal, and there was a dose-dependent and significant increase of the gene signals between 0.8 and 1.6 mM. APC and KRAS genes were more susceptible than total DNA to hexenal but only in primary colon cells.

Hexenal forms a pair of diastereomeric exocyclic 1,*N*2-propanodeoxyguanosine adducts (Hex-PdG) on reaction with deoxyguanosine [42]. These adducts have been detected in cells and tissues upon exposure to hexenal [43–45], i.e. the generated 1,*N*2-propano-dG adducts in the human lymphoblastoid Namalva cell line after exposure to 0.2 mM hexenal for 1 h amounted to 86 fmol/ḿol of DNA, and exposure to 0.4 mM for 30 min in primary rat colon mucosa cells amounted to 50 fmol/ḿol of DNA [43].

In a physiologically based in silico model for *trans*-2-hexenal detoxification and DNA adduct formation in humans including inter-individual variation Kiwamoto et al. [46] demonstrated that DNA adduct formation due to 2-hexenal exposure was 0.039 adducts/10^8 nucleotides (nt) at the estimated average 2-hexenal dietary intake of 0.04 mg 2-hexenal/kg bw and 0.18 adducts/10^8 nt at the 95th percentile of the dietary intake (0.178 mg 2-hexenal/kg bw) in the most sensitive people. Since these levels are three orders of magnitude