Mutat Res Gen Tox En 819 (2017) 14–19

Contents lists available at ScienceDirect

Mutat Res Gen Tox En

journal homepage: www.elsevier.com/locate/gentox

Short communication

Mutagenicity of ω-3 fatty acid peroxidation products in the Ames test

Petr Grúz[a,*], Masatomi Shimizu[a,b], Kei-ichi Sugiyama[a], Masamitsu Honma[a]

[a] Division of Genetics and Mutagenesis, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
[b] Division of Medical Nutrition, Faculty of Healthcare, Tokyo Healthcare University, Tokyo, Japan

ARTICLE INFO

Keywords:
Lipid peroxidation
Genotoxicity
Ames test
Omega-3 fatty acids

ABSTRACT

Polyunsaturated fatty acids (PUFA) represent one of the main building blocks of cellular membranes and their varying composition impacts lifespan as well as susceptibility to cancer and other degenerative diseases. Increased intake of ω-3 PUFA is taught to compensate for the abundance of ω-6 PUFA in modern human diet and prevent cardiocirculatory diseases. However, highly unsaturated PUFA of marine and seed origin easily oxidize to aldehydic products which form DNA adducts. With increased PUFA consumption it is prudent to re-evaluate ω-3 PUFA safety and the genotoxic hazards of their metabolites. We have used the standard Ames test to examine the mutagenicity of 2 hexenals derived from lipid peroxidation of the common ω-3 PUFA in human diet and tissues. Both 4-hydroxyhexenal and 2-hexenal derived from the ω-3 docosahexaenoic and α-linolenic acid, respectively, induced base substitutions in the TA104 and TA100 Ames strains in a dose dependent manner. Their mutagenicity was dependent on the Y-family DNA polymerase RI and they did not induce other types of mutations such as the −2 and −1 frameshifts in the TA98 and TA97 strains. Our results expand previous findings about the mutagenicity of related ω-3 peroxidation product 4-oxohexenal and raise alert that overuse of ω-3 rich oils may have adverse effect on genome stability.

1. Introduction

The main feature of polyunsaturated fatty acids (PUFA) is the presence of two or more double bonds within their acyl chain. In naturally occurring PUFA, these double bonds are separated by a single bonded −C− atom which is most susceptible to attack by reactive oxygen species such as the hydroxyl radical (Chart 1). In ω-6 PUFA, the first double bond occurs on the sixth carbon atom, while in ω-3 PUFA, the first double bond is on the third carbon atom, counting from the methyl end (denoted as ω, see Chart 1). The ω-6 and ω-3 PUFA are de-novo synthesized only in plants and cold blooded animals, such as invertebrates, as their adaptation to low temperatures. Higher organisms and endotherms can manufacture only more stable ω-9 PUFA while obtaining the ω-6 and ω-3 PUFA through diet to serve in small amounts as precursors to powerful signaling and immunomodulating molecules collectively termed eicosanoids [1,2]. As a general rule, ω-3 oppose ω-6 PUFA during the production of ω-6 derived eicosanoids which are highly stimulatory.

Over the last several decades a phobia of animal fats has led food manufacturers and health authorities to replace naturally occurring animal fats with industrially processed vegetable oils in many foods. This in turn has resulted in a massive increase in concentrated ω-6 PUFA intake in humans compared to pre-industrial populations and coincides with the epidemic of degenerative diseases characterized by chronic inflammation. To offset the proinflammatory effects of ω-6 rich diet, further ω-3 PUFA are being added to human diet to lower the ω-6 to ω-3 ratio. Although this exerts short time favorable effects by cooling down exacerbated inflammatory eicosanoid signaling and stimulate pro-apoptotic activities considered chemopreventive, it also increases the content of higly unstable peroxidation prone PUFA in the body [3].

Lipid peroxide-derived endogenous DNA adducts are considered as mediators of cancer, cardiovascular disease and neurodegeneration, the three most prevalent diseases of aging. PUFA are the direct source of lipid peroxides both *in vitro* and *in vivo*. Their susceptibility to lipid peroxidation depends on the number of active methylene groups between two double bonds within a PUFA molecule. The ω-3 fatty acids contain the highest number of double bonds and therefore produce the largest amounts of genotoxins by lipid peroxidation [1].

The short chain aldehydic products of lipid peroxidation of ω-3 (e.g. acrolein, crotonaldehyde) and ω-6 (e.g. malondialdehyde) PUFA are clearly mutagenic in the Ames test, but their longer counterparts are highly toxic to bacteria thus masking their mutagenicity. The toxicity is attributed to their preferential reactions with the sulfhydryl groups of proteins [4] which are often essential for crucial enzymatic activities. However, in the presence of a scavenger like glutathione, normally non-mutagenic compounds such as the lipid peroxidation product 4-hydro-

* Corresponding author.
E-mail address: gruz@nihs.go.jp (P. Grúz).

http://dx.doi.org/10.1016/j.mrgentox.2017.05.004
Received 24 February 2017; Received in revised form 4 May 2017; Accepted 5 May 2017
Available online 05 May 2017
1383-5718/ © 2017 Elsevier B.V. All rights reserved.

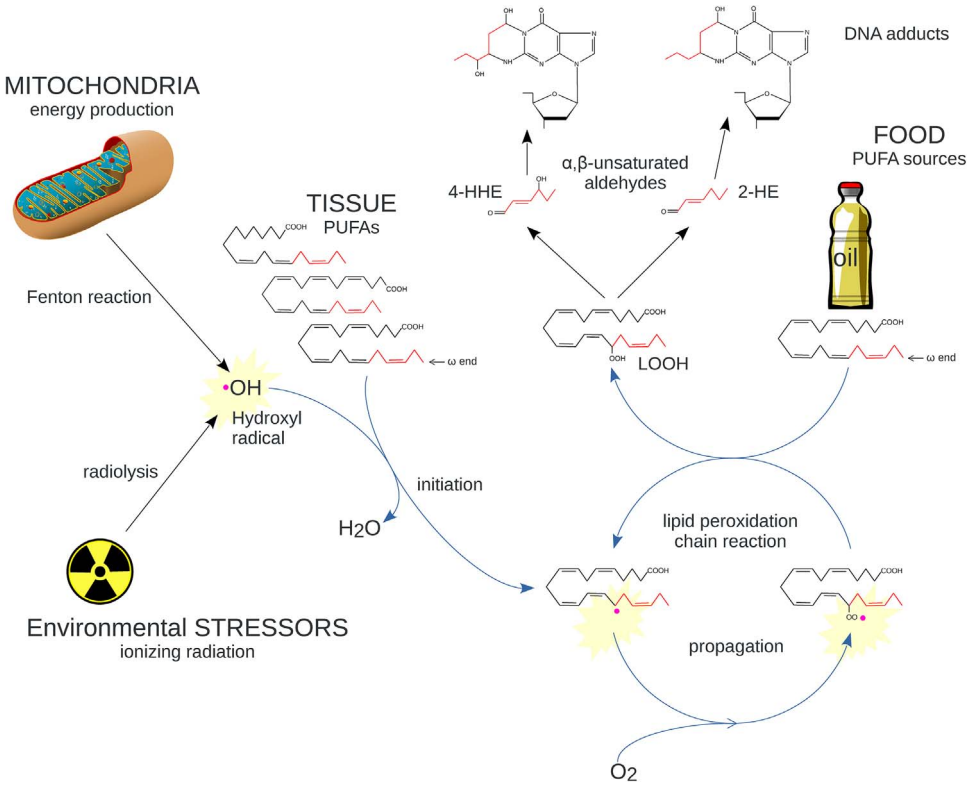

Chart 1. Pathways leading to the formation of propano-DNA adducts from hexenals. Leaks at the mitochondrial electron transport chain or water radiolysis produce ·OH radical that attacks polyunsaturated fatty acids (PUFA) containing methylene-interrupted double bonds. This triggers lipid peroxidation chain reaction that provides a continuous supply of free radicals to initiate future peroxidation with potentially devastating effects. The reaction products are lipid hydroperoxides (LOOH) which homolytically decompose to genotoxic α,β-unsaturated aldehydes.

Table 1
Ames tester strains used to screen for the mutagenicity of hexenals. Relevant genotypes and target sequences for the reversions to histidine prototrophy are indicated. Strains harboring the R-factor plasmid pKM101 expressing DNA polymerase RI (MucAB) are labelled with " + ". For the two strains which tested positive, specific mutagenicities [rev./μmol] were calculated within the linear non-toxic range at the doses of 0.4 and 1.6 μmol/plate for 4-HHE and 2-HE, respectively.

Strain	Genotype	Mutation target	DNA Pol RI	rev./μmol	
				4-HHE	2-HE
YG5144	*his*G428	TAA (base change)	−		
TA104			+	181	31
YG5151	*his*G46	GGG (base change)	−		
TA100			+	51	108
TA1537	*his*C3076	CCC (+1 frameshift)	−		
TA2637			+		
TA97	*his*D6610	CCCCCC GCGC (+1 frameshift)	+		
YG5147	*his*D3052	CGCGCGCG (-2 frameshifts)	−		
TA1538			−		
TA98			+		

xy-pentenal also become mutagens [5]. The exemplary genotoxin derived from the ω-6 dietary fatty acids is 4-hydroxynonenal (4-HNE). Despite the lack of its direct mutagenicity in bacteria, 4-HNE is a potent inducer of the DNA damage SOS response [6] and becomes a strong mutagen in both TA100 and TA104 Ames tester strains after further activation to its epoxide by e.g. reaction with peroxides [7]. Since epoxy aldehydes escape scavenging by sulfhydryl groups on proteins and glutathione, they readily react with DNA *in vivo*. The epoxy aldehydes form a variety of exocyclic etheno DNA adducts while their parental α,β-unsaturated aldehydes form the propano adducts [8]. The major propano N^2-dG DNA adducts are bypassed in an *error-free* manner with the assistance of the DinB-type DNA polymerases which may effectively lower their mutagenicity [9].

The ω-3 counterparts of the ω-6 nonenals 4-HNE and 4-ONE are the hexenals 4-HHE and 4-oxo-2-hexenal (4-OHE) which are 3 carbons in length shorter. The direct mutagenicity of 4-OHE in the Ames test has been demonstrated previously by Kasai et al. and its DNA footprint was found to constitute a significant part of the human adductome [10,11]. Although direct binding of tritium labelled 4-HHE to liver DNA has not been detected *in vivo*, 4-HHE elevated micronuclei and sister chromatid exchange formation even at non-cytotoxic levels [12]. Here we have examined the direct mutagenicity of the 4-hydroxyhexenal (4-HHE) and its cousin *trans*-2-hexenal (2-HE) in the standard set of the Ames tester strains and found that both compounds were able to induce the base substitution type mutations.

2. Materials and methods

2.1. Used chemicals

(±)-4-hydroxy-2E-hexenal (4-hydroxyhexenal, 4-HHE) and similar oxylipins were purchased from Cayman Chemical (USA), *trans*-2-hexenal (leaf aldehyde, 2-HE) and crotonaldehyde were from Wako (Japan). The AMT-S plates (Kyokuto, Japan) were used for plating in all experiments.

2.2. Bacterial strains

The tester strains used in this study were all derivatives of the *Salmonella typhimurium* LT2 differing in the type of mutations they can detect according to the mutation target sequence leading to histidine auxotrophy (Table 1). The TA strains are the original Ames test series [13,14] while the YG strains have been developed and used in our

Download English Version:

https://daneshyari.com/en/article/5528826

Download Persian Version:

https://daneshyari.com/article/5528826

Daneshyari.com