Mutation Research 786 (2016) 41–51

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Aldehydes with high and low toxicities inactivate cells by damaging distinct cellular targets

Ming-Zhang Xie[a], Mahmoud I. Shoulkamy[a,b], Amir M.H. Salem[a,c], Shunya Oba[a], Mizuki Goda[a], Toshiaki Nakano[a], Hiroshi Ide[a,*]

[a] Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Higashi-Hiroshima 739-8526, Japan
[b] Department of Zoology, Biological Science Building, Faculty of Science, Minia University, Minia 61519, Egypt
[c] Department of Pathology, Medical Research Division, National Research Centre, El-Bohouth St., Dokki, Giza 12311, Egypt

ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form 9 February 2016
Accepted 10 February 2016
Available online 15 February 2016

Keywords:
Aldehyde
Cytotoxicity
DNA damage
Protein damage
DNA repair

ABSTRACT

Aldehydes are genotoxic and cytotoxic molecules and have received considerable attention for their associations with the pathogenesis of various human diseases. In addition, exposure to anthropogenic aldehydes increases human health risks. The general mechanism of aldehyde toxicity involves adduct formation with biomolecules such as DNA and proteins. Although the genotoxic effects of aldehydes such as mutations and chromosomal aberrations are directly related to DNA damage, the role of DNA damage in the cytotoxic effects of aldehydes is poorly understood because concurrent protein damage by aldehydes has similar effects. In this study, we have analysed how saturated and α,β-unsaturated aldehydes exert cytotoxic effects through DNA and protein damage. Interestingly, DNA repair is essential for alleviating the cytotoxic effect of weakly toxic aldehydes such as saturated aldehydes but not highly toxic aldehydes such as long α,β-unsaturated aldehydes. Thus, highly toxic aldehydes inactivate cells exclusively by protein damage. Our data suggest that DNA interstrand crosslinks, but not DNA-protein crosslinks and DNA double-strand breaks, are the critical cytotoxic DNA damage induced by aldehydes. Further, we show that the depletion of intracellular glutathione and the oxidation of thioredoxin 1 partially account for the DNA damage-independent cytotoxicity of aldehydes. On the basis of these findings, we have proposed a mechanistic model of aldehyde cytotoxicity mediated by DNA and protein damage.

1. Introduction

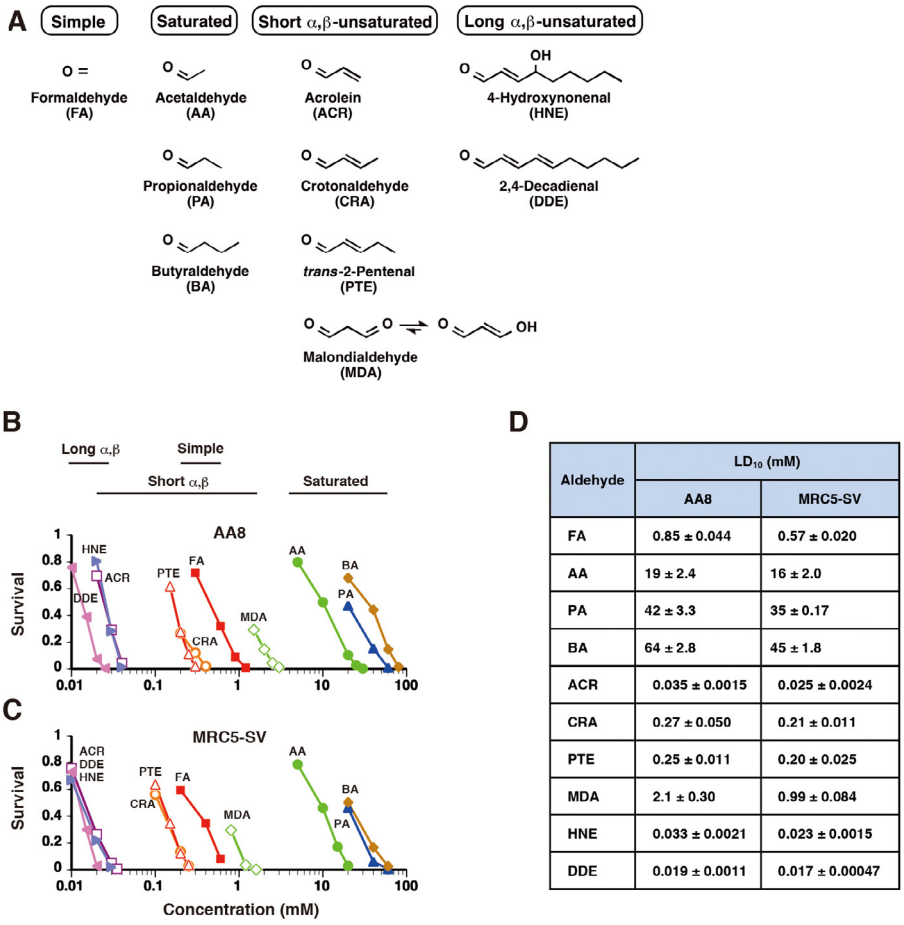
Aldehydes have long been known as genotoxic and cytotoxic molecules [1,2] and are implicated in the pathogenesis of various human diseases such as diabetes, atherosclerosis and neurological disorders [3,4]. They are produced in cells as a consequence of lipid peroxidation and autoxidation or metabolic activation of precursors [3]. In addition, exposure to anthropogenic aldehydes and intake of dietary aldehydes increases further the aldehyde burden in cells [3]. The general mechanism of aldehyde toxicity involves adduct formation with biomolecules such as DNA and proteins, resulting in the inactivation of the function of these molecules [5–7]. Thus, the cytotoxicity of aldehydes is attributed to DNA damage-dependent and DNA damage-independent mechanisms.

With DNA, aldehydes react with the exocyclic amino group of DNA bases, generating aldehyde-base adducts. Some of the aldehyde-base adducts undergo further reactions with a proximal protein or a DNA base in the opposite strand, giving rise to a DNA-protein crosslink (DPC) and a DNA interstrand crosslink (ICL), respectively. Various types of aldehyde-base adducts [8,9] and DPCs [10–12] have been identified *in vivo*. It has also been reported that aldehydes induce DNA double-strand breaks (DSBs) *in vivo* [13,14]. The formation of ICLs by aldehydes has been demonstrated *in vitro* [15], and the hypersensitivity of Fanconi anaemia (FANC) cells that are deficient in ICL repair to formaldehyde (FA) and acetaldehyde (AA) supports the formation of ICLs *in vivo* when cells are exposed to aldehydes [16,17]. The base adducts, DPCs, ICLs and DSBs stall the DNA replication fork and/or reduce the fidelity of DNA replication and transcription; therefore they can exert cytotoxic and genotoxic effects [15,18–22].

With proteins, saturated aldehydes target the lysine ε-amino group of proteins to form Schiff base adducts, and α,β-unsaturated aldehydes target the thiol (cysteine), imidazole (histidine) and ε-amino (lysine) groups of proteins to form mainly Michael addition-type adducts or Schiff base adducts [6]. If these susceptible amino acids are involved in protein function, modifications by aldehyde lead to the inactivation of the protein, hence exerting cytotoxic effects. To date, a number of enzymes involved in

* Corresponding author.
E-mail address: ideh@hiroshima-u.ac.jp (H. Ide).

http://dx.doi.org/10.1016/j.mrfmmm.2016.02.005
0027-5107/



D

Aldehyde	LD_{10} (mM)	
	AA8	MRC5-SV
FA	0.85 ± 0.044	0.57 ± 0.020
AA	19 ± 2.4	16 ± 2.0
PA	42 ± 3.3	35 ± 0.17
BA	64 ± 2.8	45 ± 1.8
ACR	0.035 ± 0.0015	0.025 ± 0.0024
CRA	0.27 ± 0.050	0.21 ± 0.011
PTE	0.25 ± 0.011	0.20 ± 0.025
MDA	2.1 ± 0.30	0.99 ± 0.084
HNE	0.033 ± 0.0021	0.023 ± 0.0015
DDE	0.019 ± 0.0011	0.017 ± 0.00047

Fig. 1. Structures, cytotoxicity and LD_{10} values of aldehydes used in this study. (A) Structures of aldehydes. (B) Survival of aldehyde-treated AA8 cells. (C) Survival of aldehyde-treated MRC5-SV cells. Cells were incubated with the indicated concentrations of aldehydes for 2 h, and survival was measured using a clonogenic assay. The data for AA8 and MRC5-SV cells are derived from the survival curves presented in Supplementary Figs. 1 and 3, respectively. In panels B and C, data points are the means of 3–5 independent experiments, and standard deviations are not shown for the clarity of plots. (D) LD_{10} values of aldehydes for AA8 and MRC5-SV cells. The values were determined graphically from survival curves in Supplementary Figs. 1 and 3. Data are the means of 3–5 independent experiments with standard deviations.

antioxidation and metabolism and proteins involved in redox signaling, transcription and cytoprotection have been identified as the targets of aldehydes [23–25]. Of these, cysteine thiol groups are suggested to be the primary nucleophilic targets of α,β-unsaturated aldehydes [25,26]. Also, aldehydes induce mitochondrial dysfunction and endoplasmic reticulum stress [1,3,27,28].

The genotoxic effects of aldehydes such as mutations and chromosomal aberrations are directly related to DNA damage. By contrast, the role of DNA damage in the cytotoxic effects of aldehydes has been poorly understood in conjunction with that of protein damage because concurrent protein damage by aldehydes has similar effects as mentioned above. To address this issue, we systematically analysed the sensitivity of cells deficient in DNA repair to various types of aldehyde. Our results reveal that cell survival against weakly toxic aldehydes heavily depends on DNA repair, whereas that against highly toxic aldehydes is independent of DNA repair. We also show that the depletion of intracellular glutathione (GSH) and the oxidation of thioredoxin (Trx) 1 partially account for the DNA damage-independent cytotoxicity of aldehydes. A mechanistic model of aldehyde cytotoxicity mediated by damage to DNA and proteins is proposed on the basis of the present findings.

2. Materials and methods

2.1. Chemicals

Propionaldehyde (PA), butyraldehyde (BA), acrolein (ACR), crotonaldehyde (CRA), *trans*-2-pentenal (PTE) and 2,4-decadienal (DDE) were purchased from Tokyo Chemical Industry. FA, AA and 4-hydroxynonenal (HNE) were obtained from Wako Pure Chemical Industry, Sigma-Aldrich and Cayman Chemical, respectively. Aldehydes were dissolved in MilliQ water (FA, AA, PA, BA, ACR and CRA), dimethyl sulfoxide (PTE and DDE) or ethanol (HNE) and used for survival assays. Malondialdehyde (MDA) was prepared before use by acid hydrolysis of 1,1,3,3-tetramethoxypropane (Tokyo Chemical Industry) [29]. The MDA concentration was determined by measuring the absorbance at 267 nm, using $\varepsilon = 31{,}800\ M^{-1}\ cm^{-1}$ [30]. Iodoacetic acid (IAA) and iodoacetamide (IAM) were purchased from Sigma-Aldrich and Tokyo Chemical Industry, respectively. Buthionine sulfoximine (BSO) was obtained from Wako Pure Chemical Industry, and auranofin and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) were obtained from Sigma-Aldrich. BSO, auranofin and CCCP were dissolved in dimethyl sulfoxide prior to use.