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Human Aldo-Keto Reductases 1B1 and 1B10: A Comparative Study on their Enzyme Activity toward Electrophilic Carbonyl Compounds

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Abstract

Aldo-keto reductase family 1 member B1 (AKR1B1, 1B1 in brief) and aldo-keto reductase family 1 member B10 (AKR1B10, 1B10 in brief) are two proteins with high similarities in their amino acid sequences, stereo structures, and substrate specificity. However, these two proteins exhibit distinct tissue distributions; 1B10 is primarily expressed in the gastrointestinal tract and adrenal gland, whereas 1B1 is ubiquitously present in all tissues/organs, suggesting their difference in biological functions. This study evaluated in parallel the enzyme activity of 1B1 and 1B10 toward alpha, beta-unsaturated carbonyl compounds with cellular and dietary origins, including acrolein, crotonaldehyde, 4-hydroxynonenal, trans-2-hexenal, and trans-2,4-hexadienal. Our results showed that 1B10 had much better enzyme activity and turnover rates toward these chemicals than 1B1. By detecting the enzymatic products using high-performance liquid chromatography, we measured their activity to carbonyl compounds at low concentrations. Our data showed that 1B10 efficiently reduced the tested carbonyl compounds at physiological levels, but 1B1 was less effective. Ectopically expressed 1B10 in 293T cells effectively eliminated 4-hydroxynonenal at 5 μ M by reducing to 1, 4-dihydroxynonene, whereas endogenously expressed 1B1 did not. The 1B1 and 1B10 both showed enzyme activity to glutathione-conjugated carbonyl compounds, but 1B1 appeared more active in general. Together our data suggests that 1B10 is more effectual in eliminating free electrophilic carbonyl compounds, but 1B1 seems more important in the further detoxification of glutathione-conjugated carbonyl compounds.

Keywords

Aldo-keto reductase family 1 member B1; aldo-keto reductase family 1 member B10; electrophilic carbonyl compounds; glutathione-carbonyls; and enzyme kinetics

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1. Introduction

Aldo-keto reductase (AKR) superfamily consists of more than 100 members, categorized into a hierarchy of functionally/evolutionarily related families (<40% amino acid identity with other families) and subfamilies (>60% identity among constituent members) [1, 2]. Protein enzymes in this superfamily share structural similarities and substrate specificity, and are widely involved in xenobiotic detoxification, osmotic regulation, hormonal metabolism, fatty acid and lipid synthesis, diabetic complications, procarcinogen activation, and cancer therapeutics [3–11]. In humans, aldo-keto reductase family 1 member B1 (AKR1B1, 1B1 in brief; also known as aldose reductase, AR) and aldo-keto reductase family 1 member B10 (AKR1B10, 1B10 in brief; also designated aldose reductase-like-1, ARL-1) are two homologues of AKR family 1 subfamily B (AKR1B) in humans [12, 13]. These two proteins share similar 3D structures and substrate spectra, but display distinct tissue distributions. 1B1 is ubiquitously expressed in almost all tissues, whereas 1B10 is enriched in gastrointestinal tract and adrenal gland [12, 14], suggesting their differences in biological functions.

The 1B1 and 1B10 both are monomeric NADPH-dependent enzymes with activity toward a range of xenobiotics, reducing their carbonyl groups to alcoholic forms. These compounds include electrophilic lipid peroxides [12, 15–18], retinals (all-*trans*-retinal, 9-*cis*-retinal, and 13-*cis*-retinal) [19, 20], cigarette and environmental pro-carcinogen polycyclic aromatic hydrocarbons (PAH) [21], and cytostatic anticancer agents such as daunorubicin [8, 22–24]. Among 1B1 and 1B10's substrates, alpha, beta-unsaturated carbonyl compounds may be of more important pathophysiological significance due to their high carcinogenicity and human exposures on a daily basis. These carbonyl compounds are constantly produced intracellularly during the metabolism of lipids, carbohydrates, amino acids, biogenic amines, vitamins, and steroids, particularly in oxidative stress [25–27]. For instance, 4-hydroxynonenal (HNE) in tissues is 0.1 to 3.0 μM at physiological conditions, but increases up to $\sim 10 \mu\text{M}$ under oxidative stress [28]. Humans are also exposed to carbonyl threats via daily food and drink consumption [29–31]. For example, crotonaldehyde exists in vegetables (1.4–100 $\mu\text{g/kg}$), fruits (5.4–78 $\mu\text{g/kg}$), fish (71.4–1000 $\mu\text{g/kg}$), meat (10–270 $\mu\text{g/kg}$), and alcoholic beverages such as wine (300–700 $\mu\text{g/l}$) and whisky (30–210 $\mu\text{g/l}$) [30]. The daily exposure of humans to trans-2-hexenal is up to $\sim 350 \mu\text{g}$ [31]. In addition, electrophilic carbonyl compounds are also produced by microbes in the lumen of gastrointestinal (GI) tract, such as acetaldehyde derived from alcohol consumption. Therefore, the human GI tract where both 1B1 and 1B10 are expressed is a major organ exposed to carbonyl threats. This study focused on investigating 1B1 and 1B10's enzyme activity to the alpha, beta-unsaturated carbonyl compounds with cellular and dietary-origins to understand their defensive role against carbonyl lesions in the human GI tract.

2. Material and methods

2.1 Chemicals

Reduced glutathione, acrolein, crotonaldehyde, trans-2-hexenal, trans-2, 4-hexadienal, NADPH, acetonitrile, β -mercaptoethanol (β -ME), and formic acid were purchased from Sigma, MO. HNE was produced by hydrochloric acid hydrolysis of 4-hydroxynonenal-dimethylacetal (Sigma, MO) as previously described [32]. Penicillin, streptomycin, fetal bovine serum (FBS), trypsin, and DMEM were purchased from Invitrogen, CA.

2.2 Cell culture

Transformed human embryonic kidney cells 293T (American Type Culture Collection, VA) were maintained in DMEM supplemented with 10% FBS, 100 μ /ml penicillin and 100 μ g/ml streptomycin in a humidified incubator containing 5% CO₂ at 37°C.

2.3 EGFP-AKR1B10 expression vector and transient transfection

Enhanced green fluorescent protein (EGFP)-AKR1B10 expression construct and EGFP-C3 control vector (Promega, WI) were prepared as described previously [33]. The 293T cells (3×10^5 cells/well) were seeded in six-well plates and transient transfection was performed using ExGen-500 (Fermentas, MD), following manufacturer's instructions. Expression and functionality of EGFP-AKR1B10 fusion protein were verified by Western blot and enzymatic activity as previously described [33]. EGFP-C3 vector was introduced into 293T cells in parallel as a control.

2.4 Intracellular metabolism of HNE

The 293T cells transfected with the EGFP-AKR1B10 or control vector EGFP-C3 were collected by trypsinization after incubation for 36 hours. Trypsin was neutralized by a complete medium containing 10% FBS. After being washed by PBS, cells were resuspended at 1.0×10^7 cells/ml in a serum-free DMEM containing 1.0 or 5.0 μ M HNE. At indicated time points, 40 μ l of the cell suspension (4.0×10^5 cells) was transferred into a tube containing 20 μ l of perchloric acid (45%), vortexed immediately, and then kept on ice. Aqueous phase was collected by centrifugation at 14,000 rpm, 4°C for 10 min, and 30 μ l was used for high-performance liquid chromatography (HPLC) analysis. The assays were run in triplicate for statistical significance.

2.5 HPLC procedures

A HPLC system equipped with a dual UV detector (Shimadzu, Japan) was used. A Premier C18 column (4.0 $\times$ 250mm) with 5 μ m particles, preceded by a pre-column with the same materials, was utilized for separations. Mobile phase was a mixture of Buffer A (0.2%, v/v, formic acid in deionized water) and Buffer B (acetonitrile; Sigma, MO). Separation and detecting conditions are summarized in Table S1 in Supplementary Data.

2.6 Western blot

Cell lysis, protein (50 μ g) separation and blotting were performed as previously described [7]. After blockage with 5% skim milk in PBS at room temperature for 60 min, membranes were incubated overnight with 1B1 or 1B10 antibodies (1:500) generated in our laboratory, followed by incubation with goat anti-rabbit IgG (1:2000) for 1 hour. Antibody binding was detected using an enhanced chemiluminescence system (Pierce, Rockford, IL). A β -Actin monoclonal antibody (1:40,000; Sigma, MO) was used to correct protein amounts.

2.7 Purification of 1B1 and 1B10 recombinant proteins and enzyme activity assays

1B1 and 1B10 recombinant proteins were prepared using a pQE prokaryotic protein expression system (Qiagen, CA) as previously described [33]. Enzyme activity was measured at 35°C for 10 min in 1 ml of the reaction mixture containing 125 mM sodium phosphate (pH 7.0 for 1B10 or pH 6.4 for 1B1), 50 mM KCl for 1B10/0.4 M Li₂SO₄ for 1B1, 200 μ M NADPH, 2 μ g purified protein, and appropriate substrates. D, L-glyceraldehyde was used as a standard to monitor the enzymatic activity of purified proteins. Protein-free blank controls were included. In kinetic assays with higher substrate concentrations, the decrease of NADPH was monitored by a spectrophotometer at 340 nm to indicate enzyme activity, presented as oxidized NADPH nmol/mg/min protein. Otherwise, enzymatic products were quantitatively measured by HPLC. Michaelis-Menten constants

(K_m and V_{max}) were calculated with GraphPad Prism 4 (Graph Pad Software, CA). $k_{cat} = V_{max}/[E]$. [E] denotes enzyme concentrations in molar.

2.8 Synthesis of glutathione conjugates

Glutathione conjugates of carbonyl compounds (GS-carbonyls) were synthesized by incubating 3 mM glutathione with 600 μ M of acrolein, crotonaldehyde, HNE, trans-2-hexenal, or trans-2, 4-hexadienal in 100 mM sodium phosphate (pH 7.0) at room temperature for 90 min. Progress of the reactions was monitored by the decrease in absorbance A_{210} for acrolein, A_{220} for crotonaldehyde, A_{223} for HNE, A_{225} for trans-2-hexenal, and A_{278} for trans-2, 4-hexadienal. Resulting products were purified by HPLC with a Premier C18 column (Shimadzu, Japan) and confirmed by high-performance liquid chromatography-mass spectroscopy (HPLC-MS). Amounts of formed conjugates were calculated by quantitating residual carbonyl compounds left considering a 1:1 molar ratio conjugation between glutathione and carbonyl compounds. Reduced GS-carbonyl standards were prepared by complete reduction with excessive AKR1B1 proteins (50 μ g).

2.9 HPLC-MS procedures

GS-carbonyl conjugates and enzymatic products were analyzed by an injection of one microliter of each sample into a ThermoFinnigan Surveyor HPLC equipped with a Gemini® narrow bore column (150 $\times$ 20 mm) packed with 3 μ M C₁₈ (110Å) at a flow rate of 0.150 ml/min of a binary mobile phase gradient at 50:50 (A:B, Δ 2.5%) in 20 ml (mobile phase A = 0.01% formic acid; mobile phase B = acetonitrile) until the proper peak shape, and separation and reduction of interferences were obtained. A ThermoFinnigan (TSQ7000) triple stage quadrupole (TSQ) mass spectrometer equipped with an electrospray ionization source (ESI) was calibrated with MRFA (L-methionyl-arginyl-phenylalanyl-alanine acetate) for both the single and double charge state (m/z 524.2 and 262.6, respectively) to provide a 0.1 amu mass accuracy for each $[M+H]^+$ parent ion (m_p^{++}). A full scan collection in a mass range from 150–600 m/z through a quadrupole filter (Q1) for each substrate/product of interest, e.g. m/z 406.1 for GS-trans-2, 4-hexadienol, was obtained with a capillary temperature of 250°C maintained at 4.5 kV. The m_p^{++} ions from Q1 were passed through a collision chamber (Q2), operating in a radio-frequency-only mode, and subsequently scanned through a third mass filter (Q3).

2.10 Statistical analysis

Student *t* tests, or Chi-square tests of independence as appropriate, were used for statistically significant tests of the data with $p < 0.05$.

3. Results

3.1 Enzyme activity of 1B1 and 1B10 proteins to α,β -unsaturated carbonyls

3.1.1 Preparation of 1B1 and 1B10 recombinant proteins—With a Qiagen prokaryotic protein expression system, 1B1 and 1B10 recombinant proteins were purified in parallel for *in vitro* enzymatic assays. In this system, a 6x histidine tag was added at N-terminus for protein purification. As exhibited in Figure 1A, a single protein band (approximately 36.0 kDa) was detected by Coomassie blue staining, indicating the purity of the prepared 1B1 and 1B10 proteins.

3.1.2 Enzyme kinetic properties of 1B1 and 1B10 recombinant proteins—The 1B1 and 1B10 are both active toward a range of xenobiotic carbonyl compounds, reducing the carbonyl groups into hydroxide forms with NADPH as a hydrogen donor, but the data from different laboratories were less comparative because of the variations in protein

preparation and enzyme reaction conditions, which often significantly affect the results. In this study, we performed in parallel the enzymatic activity assays for both 1B1 and 1B10. Alpha, beta-unsaturated carbonyl compounds to which humans are exposed daily were chosen as substrates, including acrolein, crotonaldehyde, HNE, trans-2-hexenal, and trans-2,4-hexadienal. As shown in Figure 1B, 1B1 and 1B10 both demonstrated a Michaelis-Menten kinetics to HNE, but 1B1 had a markedly lower enzyme activity and product turnover rates than 1B10. All tested carbonyl compounds exhibited a steady-state kinetic property, and Table 1 summarizes the kinetic constants.

3.1.3 Enzymatic activity of 1B1 and 1B10 toward alpha, beta-unsaturated carbonyl compounds at low levels—Human physiological exposures to carbonyl compounds are usually at low levels; however, there is not a threshold for the cytotoxicity and genotoxicity of carbonyl compounds. Reactive carbonyl compounds need to be eliminated efficiently to prevent any far-reaching effects, such as cumulative DNA mutations in particular. To understand their detoxicant role in physiological conditions, we tested the enzyme activity of 1B1 and 1B10 towards carbonyl compounds at low concentrations via HPLC analysis of the enzymatic products. Figure 2 shows the HPLC data of the reduction products of HNE and trans-2,4-hexadienal by 1B1 and 1B10. As summarized in Table 2, 1B10 showed a much higher enzyme activity to the tested carbonyl compounds than 1B1, suggesting its importance in preventing alpha, beta-unsaturated carbonyl compounds at physiological conditions.

3.1.4 Ectopically expressed 1B10 efficiently eliminates HNE in 293T cells—To verify the role of 1B10 in carbonyl elimination, we conducted an intracellular study. An EGFP-AKR1B10 fusion protein was transiently delivered into 293T cells that express 1B1, but not 1B10. This EGFP-AKR1B10 fusion protein (1B10 plus EGFP $\approx$ 65.0 kDa) was detected by Western blot (Figure 3A) and yielded $\sim$ 4-fold increase of enzyme activity to DL-glyceraldehyde (Figure 3B), a substrate for both 1B1 and 1B10 [12], indicating that ectopically expressed EGFP-AKR1B10 is enzymatically functional.

Intracellular metabolism of HNE was evaluated by measuring the remaining HNE and its reduction product DHN by HPLC at different time points. As demonstrated in Figure 3C, in the 293T cells expressing the EGFP-AKR1B10 fusion protein, HNE was rapidly cleared up by reduction to DHN, whereas DHN was not detected in the vector control cells in which 1B1 was expressed and enzymatically active (Figure 3B). This intracellular data supports the *in vitro* enzyme activity results of recombinant proteins.

3.2 Enzymatic activity of 1B1 and 1B10 toward GS-carbonyl conjugates

3.2.1 Synthesis of GS-carbonyl conjugates—GS-carbonyls were chemically synthesized, purified by HPLC, and verified by LC-MS. Figure 4 shows the mass spectral data of GS-butanal, GS-hexanal, and GS-4-hydroxynonanal. Conjugation of HNE to glutathione produces two chiral carbon atoms and subsequent ring closure of GS-4-hydroxynonanal, giving rise to different diastereoisomers [34]. In this study, GS-4-hydroxynonanal appeared as three peaks (Figure 4C) with the same m/z 464.2. Similarly, GS-propanal and GS-trans-4-hexenal were chemically synthesized, purified by HPLC and characterized with LC-MS (data not shown).

3.2.2 1B1 and 1B10 enzyme activity toward GS-carbonyl conjugates—Inside the cells, glutathione-s-transferases catalyze the conjugation of carbonyl compounds with glutathione as one of the metabolic pathways; 1B1 catalyzes the reduction of GS-carbonyls for further detoxification [35, 36]. For a comparison, enzymatic activities of 1B1 and 1B10 to GS-carbonyls were measured in parallel; the enzymatic products were analyzed by HPLC

and verified by LC-MS. Figure 5 shows the reduction products of GS-hexanal and GS-trans-4-hexenal by 1B1 and 1B10. The data showed that 1B1 had a higher enzyme activity to GS-carbonyls except for GS-trans-4-hexenal with two unsaturated carbon bonds (Table 1). GS-4-hydroxynonanal with a chain of 9 carbons was not an appreciable substrate for 1B10.

4. Discussion

Human 1B1 and 1B10 in AKR1B subfamily were extensively investigated in terms of their tissue distributions, crystal structures, substrate specificity, and S-thiolation modifications [4, 5, 12, 20, 37, 38], but a distinctive study on the physiological xenobiotics is lacking. This study focused on 1B1 and 1B10's enzyme activity to pathophysiologically important alpha, beta-unsaturated carbonyl-compounds: acrolein, crotonaldehyde, HNE, trans-2-hexenal, and trans-2,4-hexadienal. Through a parallel comparison, we recognized a differential activity of 1B1 and 1B10 in eliminating cytotoxic carbonyl compounds.

Alpha, beta-unsaturated carbonyl compounds are generated intracellularly by lipid peroxidation, ingested from various foodstuffs and beverages, and produced by luminal microbes; they are highly electrophilic with strong cytotoxicity, mutagenicity, and carcinogenicity [25–27]. Therefore, it is important to understand the defensive mechanisms of humans, particularly for their GI tract with daily carbonyl threats from diets and luminal microbes. 1B1 and 1B10 are both expressed in the GI tract, and thus we investigated and compared their enzyme activity to the alpha, beta-unsaturated carbonyl compounds indicated above. Our results showed that 1B10 had a much higher enzyme activity and turnover rates to the free carbonyl compounds, but 1B1 appeared more appreciable to GS-conjugated carbonyl-compounds, particularly for GS-4-hydroxynonanal. As shown in Table.1, the catalytic activity of 1B10 progressively decreased with the increase of the carbon chain length in GS-conjugated carbonyl compounds, whereas 1B1's activity increased with carbon chain length. This differential activity of 1B1 and 1B10 to free and glutathione-conjugated carbonyl compounds may indicate a different structure-function relationship in these two enzyme proteins. An addition of a side chain (glutathione) may alter the 'fit' of the carbonyl conjugates with a long carbon chain into the active pocket of 1B10, but not into 1B1's. It is noteworthy that 1B10 was less active to acrolein at low concentrations. This may be attributed to the suboptimal enzymatic conditions. Due to the difficulty in separating acrolein reduction products, allyl alcohol from β -mercaptoethanol (β -ME) by HPLC, 1B10 protein for acrolein assays was prepared without β -ME, which led to more than a three fold decrease in its activity to DL-glyceraldehyde (data not shown). The same mechanism may be applicable to explain the lower 1B1 activity observed in this study than that reported previously. In this study, the mild sulphydryl reducer, β -ME (10 mM) was used in protein preparation, but dithiothreitol-reduction of 1B1 was not conducted before the enzyme activity assay in order to make the *in vitro* data more similar to physiological conditions. Indeed, this enzymatic data fitted well with that from the intracellular study. In 293T cells, ectopically expressed EGFP-AKR1B10 efficiently converted HNE at 5 μ M to DHN, but the endogenously expressed 1B1, although active to DL-glyceraldehyde, did not. As a result, DHN was barely detectable in the vector control 293T cells. In this study, serum-free cell suspensions were used to exclude serum-derived artifacts that may affect HNE metabolism.

AKR1B10 is highly induced in human tumors [12, 13]. The findings in this study may help to understand the pathophysiological role of 1B10 in cancer development and progression. Cancer cells grow aggressively and are metabolically active, thus having a high burden of toxic carbonyl compounds. 1B10 upregulated in cancer cells may facilitate the cell growth and proliferation via eliminating the toxic carbonyl compounds [33, 37].

In summary, 1B1 and 1B10 are both expressed in the human GI tract, but have differential substrate specificity on free or GS-conjugated carbonyl compounds, suggesting their distinct roles against carbonyl lesions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations used

AKR1B1	aldo-keto reductase family 1 member B1
AKR1B10	aldo-keto reductase family 1 member B10
BME	beta-mercaptoethanol
DHN	1,4-dihydroxynonene
GSH	glutathione
HNE-DMA	4-hydroxynonenal-dimethylacetal
HPLC	high-performance liquid chromatography
NADPH	β -Nicotinamide adenine dinucleotide 2'-phosphate reduced
PAH	polycyclic aromatic hydrocarbon

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References

1. Mindnich RD, Penning TM. Aldo-keto reductase (AKR) superfamily: genomics and annotation. *Hum Genomics*. 2009; 3(4):362–370. [PubMed: 19706366]
2. Jez JM, Flynn TG, Penning TM. A new nomenclature for the aldo-keto reductase superfamily. *Biochemical pharmacology*. 1997; 54(6):639–647. [PubMed: 9310340]
3. Barski OA, Tipparaju SM, Bhatnagar A. The aldo-keto reductase superfamily and its role in drug metabolism and detoxification. *Drug Metab Rev*. 2008; 40(4):553–624. [PubMed: 18949601]
4. Petrash JM. All in the family: aldose reductase and closely related aldo-keto reductases. *Cell Mol Life Sci*. 2004; 61(7–8):737–749. [PubMed: 15094999]
5. Jin Y, Penning TM. Aldo-keto reductases and bioactivation/detoxication. *Annual review of pharmacology and toxicology*. 2007; 47:263–292.
6. Yasuda N, Fujino K, Shiraji T, Nambu F, Kondo K. Effects of steroid 5 α -reductase inhibitor ONO-9302 and anti-androgen allylestrenol on the prostatic growth, and plasma and prostatic hormone levels in rats. *Japanese journal of pharmacology*. 1997; 74(3):243–252. [PubMed: 9268084]
7. Jin J, Krishack PA, Cao D. Role of aldo-keto reductases in development of prostate and breast cancer. *Front Biosci*. 2006; 11:2767–2773. [PubMed: 16720349]
8. Lee KW, Ko BC, Jiang Z, Cao D, Chung SS. Overexpression of aldose reductase in liver cancers may contribute to drug resistance. *Anticancer Drugs*. 2001; 12(2):129–132. [PubMed: 11261885]
9. Wang C, Yan R, Luo D, Watabe K, Liao DF, Cao D. Aldo-keto reductase family 1 member B10 promotes cell survival by regulating lipid synthesis and eliminating carbonyls. *J Biol Chem*. 2009; 284(39):26742–26748. [PubMed: 19643728]

10. Ma J, Yan R, Zu X, Cheng JM, Rao K, Liao DF, Cao D. Aldo-keto reductase family 1 B10 affects fatty acid synthesis by regulating the stability of acetyl-CoA carboxylase- α in breast cancer cells. *J Biol Chem*. 2008; 283(6):3418–3423. [PubMed: 18056116]
11. Tammali R, Reddy AB, Ramana KV, Petrash JM, Srivastava SK. Aldose reductase deficiency in mice prevents azoxymethane-induced colonic preneoplastic aberrant crypt foci formation. *Carcinogenesis*. 2009; 30(5):799–807. [PubMed: 19028703]
12. Cao D, Fan ST, Chung SS. Identification and characterization of a novel human aldose reductase-like gene. *J Biol Chem*. 1998; 273(19):11429–11435. [PubMed: 9565553]
13. Chung S, LaMendola J. Cloning and sequence determination of human placental aldose reductase gene. *J Biol Chem*. 1989; 264(25):14775–14777. [PubMed: 2504709]
14. Hyndman DJ, Flynn TG. Sequence and expression levels in human tissues of a new member of the aldo-keto reductase family. *Biochimica et biophysica acta*. 1998; 1399(2–3):198–202. [PubMed: 9765596]
15. Aldini G, Dalle-Donne I, Facino RM, Milzani A, Carini M. Intervention strategies to inhibit protein carbonylation by lipoxidation-derived reactive carbonyls. *Medicinal research reviews*. 2007; 27(6):817–868. [PubMed: 17044003]
16. Zhong L, Liu Z, Yan R, Johnson S, Zhao Y, Fang X, Cao D. Aldo-keto reductase family 1 B10 protein detoxifies dietary and lipid-derived α , β -unsaturated carbonyls at physiological levels. *Biochem Biophys Res Commun*. 2009; 387(2):245–250. [PubMed: 19563777]
17. Spite M, Baba SP, Ahmed Y, Barski OA, Nijhawan K, Petrash JM, Bhatnagar A, Srivastava S. Substrate specificity and catalytic efficiency of aldo-keto reductases with phospholipid aldehydes. *The Biochemical journal*. 2007; 405(1):95–105. [PubMed: 17381426]
18. Martin HJ, Maser E. Role of human aldo-keto-reductase AKR1B10 in the protection against toxic aldehydes. *Chem Biol Interact*. 2009; 178(1–3):145–150. [PubMed: 19013440]
19. Ruiz FX, Gallego O, Ardevol A, Moro A, Dominguez M, Alvarez S, Alvarez R, de Lera AR, Rovira C, Fita I, Pares X, Farres J. Aldo-keto reductases from the AKR1B subfamily: retinoid specificity and control of cellular retinoic acid levels. *Chemico-biological interactions*. 2009; 178(1–3):171–177. [PubMed: 19014918]
20. Gallego O, Ruiz FX, Ardevol A, Dominguez M, Alvarez R, de Lera AR, Rovira C, Farres J, Fita I, Pares X. Structural basis for the high all-trans-retinaldehyde reductase activity of the tumor marker AKR1B10. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(52):20764–20769. [PubMed: 18087047]
21. Quinn AM, Harvey RG, Penning TM. Oxidation of PAH trans-dihydrodiols by human aldo-keto reductase AKR1B10. *Chemical research in toxicology*. 2008; 21(11):2207–2215. [PubMed: 18788756]
22. Martin HJ, Breyer-Pfaff U, Wsol V, Venz S, Block S, Maser E. Purification and characterization of akr1b10 from human liver: role in carbonyl reduction of xenobiotics. *Drug Metab Dispos*. 2006; 34(3):464–470. [PubMed: 16381663]
23. Balendiran GK, Martin HJ, El-Hawari Y, Maser E. Cancer biomarker AKR1B10 and carbonyl metabolism. *Chem Biol Interact*. 2009; 178(1–3):134–137. [PubMed: 19028477]
24. Balendiran GK. Fibrates in the chemical action of daunorubicin. *Curr Cancer Drug Targets*. 2009; 9(3):366–369. [PubMed: 19442055]
25. De Bont R, van Larebeke N. Endogenous DNA damage in humans: a review of quantitative data. *Mutagenesis*. 2004; 19(3):169–185. [PubMed: 15123782]
26. Davydov VV, Dobaeva NM, Bozhkov AI. Possible role of alteration of aldehyde's scavenger enzymes during aging. *Experimental gerontology*. 2004; 39(1):11–16. [PubMed: 14724059]
27. Berhane K, Widersten M, Engstrom A, Kozarich JW, Mannervik B. Detoxication of base propenals and other α , β -unsaturated aldehyde products of radical reactions and lipid peroxidation by human glutathione transferases. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91(4):1480–1484. [PubMed: 8108434]
28. Esterbauer H, Eckl P, Ortner A. Possible mutagens derived from lipids and lipid precursors. *Mutation research*. 1990; 238(3):223–233. [PubMed: 2342513]
29. Wang GW, Guo Y, Vondriska TM, Zhang J, Zhang S, Tsai LL, Zong NC, Bolli R, Bhatnagar A, Prabhu SD. Acrolein consumption exacerbates myocardial ischemic injury and blocks nitric oxide-

- induced PKCepsilon signaling and cardioprotection. *J Mol Cell Cardiol.* 2008; 44(6):1016–1022. [PubMed: 18468618]
30. Schuler BD, Eder E. Development of a 32P-postlabelling method for the detection of 1,N2-propanodeoxyguanosine adducts of crotonaldehyde in vivo. *Arch Toxicol.* 2000; 74(7):404–414. [PubMed: 11043496]
31. Stout MD, Bodes E, Schoonhoven R, Upton PB, Travlos GS, Swenberg JA. Toxicity, DNA binding, and cell proliferation in male F344 rats following short-term gavage exposures to trans-2-hexenal. *Toxicologic pathology.* 2008; 36(2):232–246. [PubMed: 18362197]
32. Srivastava S, Watowich SJ, Petrash JM, Srivastava SK, Bhatnagar A. Structural and kinetic determinants of aldehyde reduction by aldose reductase. *Biochemistry.* 1999; 38(1):42–54. [PubMed: 9890881]
33. Zu X, Yan R, Robbins S, Krishack PA, Liao DF, Cao D. Reduced 293T cell susceptibility to acrolein due to aldose reductase-like-1 protein expression. *Toxicol Sci.* 2007; 97(2):562–568. [PubMed: 17329238]
34. Cheng JZ, Sharma R, Yang Y, Singhal SS, Sharma A, Saini MK, Singh SV, Zimniak P, Awasthi S, Awasthi YC. Accelerated metabolism and exclusion of 4-hydroxynonenal through induction of RLIP76 and hGST5.8 is an early adaptive response of cells to heat and oxidative stress. *The Journal of biological chemistry.* 2001; 276(44):41213–41223. [PubMed: 11522795]
35. Coles BF, Kadlubar FF. Detoxification of electrophilic compounds by glutathione S-transferase catalysis: determinants of individual response to chemical carcinogens and chemotherapeutic drugs? *BioFactors (Oxford, England).* 2003; 17(1–4):115–130.
36. Srivastava S, Chandra A, Bhatnagar A, Srivastava SK, Ansari NH. Lipid peroxidation product, 4-hydroxynonenal and its conjugate with GSH are excellent substrates of bovine lens aldose reductase. *Biochemical and biophysical research communications.* 1995; 217(3):741–746. [PubMed: 8554593]
37. Yan R, Zu X, Ma J, Liu Z, Adeyanju M, Cao D. Aldo-keto reductase family 1 B10 gene silencing results in growth inhibition of colorectal cancer cells: Implication for cancer intervention. *Int J Cancer.* 2007; 121(10):2301–2306. [PubMed: 17597105]
38. Shen Y, Zhong L, Markwell S, Cao D. Thiol-disulfide exchanges modulate aldo-keto reductase family 1 member B10 activity and sensitivity to inhibitors. *Biochimie.* 2010; 92(5):530–537. [PubMed: 20144905]

Fig. 1. AKR1B1 and AKR1B10 proteins and Substrate-Velocity curves of HNE

1B1 and 1B10 protein preparation and enzyme activities were conducted as described in the Materials and Methods. *A*) AKR1B1 (1B1) and AKR1B10 (1B10) recombinant proteins, displayed by Coomassie blue staining. *B*) Substrate-Velocity curves and enzyme kinetic constants of HNE, produced with GraphPad Prism 4 (Graph Pad Software, CA). HNE, 4-hydroxynonenal.

A) 4-Hydroxynonenal

i) AKR1B10

ii) AKR1B1

B) Trans-2, 4-hexadienal

i) AKR1B10

ii) AKR1B1

Fig. 2. AKR1B1 and AKR1B10 activity to carbonyl compounds at low concentrations

Enzyme reactions and HPLC analysis of the products were performed as described in Materials and Methods. Substrates were used at low concentrations as indicated on the right. Fifty microliters were used for the HPLC analysis. Peaks (labeled) are displayed in the same scales.

A) Western blot

B) AKR1B10 activity

C) HNE (5 μ M) metabolism in 293T cells

i) EGFP-AKR1B10 cells

ii) EGFP vector control cells

Fig. 3. HNE elimination by ectopically expressed AKR1B10 in 293T cells

AKR1B10 protein fused to the C-terminus of EGFP (EGFP-AKR1B10) was transiently expressed in 293T cells as described in the Materials and Methods. A) Western blot for EGFP-AKR1B10 and endogenous AKR1B1. B) Enzyme activity (oxidized NADPH at nmol/mg protein/hour). * $p < 0.01$, compared to vector control cells. C) Metabolism of HNE (5 μ M) in 293T cells. (i) 293T cells transfected with EGFP-AKR1B10 and (ii) 293T cells transfected with EGFP vector. HNE, 4-hydroxynonenal; DHN, 1, 4-dihydroxynonene.

A) GS-butanal

B) GS-hexanal

C) GS-4-hydroxynonanal

Fig. 4. Glutathione-conjugated carbonyl compounds

GS-carbonyls were purified by HPLC and then subjected to full scan mass-spectra (m/z 150–600) as described in the Materials and Methods. A) GS-butanal with an $[M+H]^+$ parent ion (m_p^+) at 378.00. B) GS-hexenal with an $[M+H]^+$ parent ion (m_p^+) at 408.12. C) GS-4-hydroxynonanal with an $[M+H]^+$ parent ion (m_p^+) at 464.2. In the dot frame, total ion chromatogram (TIC) shows three peaks of different chiral forms of GS-4-hydroxynonanal.

Fig. 5. AKR1B1 and AKR1B10 activity toward GS-hexenal and GS-trans-4-hexenal

A) HPLC analysis. Enzymatic reactions and HPLC analysis were conducted as described in the Materials and Methods. GS-hexenal and GS-trans-4-hexenal were used at the low concentrations as indicated on the right. Fifty microliters were used for the HPLC analysis. **(B)** Full scans mass spectra (m/z 150–600). GS-hexenal and GS-trans-4-hexenal have $[M + H]^+$ 408.1 and 406.1, respectively. Data was modified from Zhong et al. (2009)

Table 1
Kinetic parameters of AKR1B1 and AKR1B10 to carbonyl compounds and glutathione conjugates

AKR1B10 and AKR1B1 activity were measured as described in Materials and Methods. Constants were calculated by a Lineweaver-Burk plot of the reduction rate at various substrate concentrations. GS, glutathione; ND, no activity was detected when 0.1 – 0.5 μ M of GS-4-hydroxynonenal was used in the enzyme activity assays with 2 μ g AKR1B10. Data was modified from Zhong *et al.* (2009)

	C_n	1B1			1B10		
		K_m (mM)	k_{cat} (min ⁻¹)	k_{cat}/K_m (min ⁻¹ mM ⁻¹)	K_m (mM)	k_{cat} (min ⁻¹)	k_{cat}/K_m (min ⁻¹ mM ⁻¹)
D,L-Glyceraldehyde	3	0.065 $\pm$ 0.010	33	507	0.563 $\pm$ 0.045	29	52
Acrolein	3	0.884 $\pm$ 0.280	11	12	0.110 $\pm$ 0.012	116	1070
Crotonaldehyde	4	9.643 $\pm$ 0.929	31	3	0.086 $\pm$ 0.014	103	1200
Trans-2-hexenal	6	0.878 $\pm$ 0.151	41	47	0.061 $\pm$ 0.019	96	1580
Trans-2, 4-hexadienal	6	0.905 $\pm$ 0.299	43	47	0.096 $\pm$ 0.056	78	813
4-Hydroxynonenal	9	0.716 $\pm$ 0.046	50	70	0.031 $\pm$ 0.007	119	3839
GS-propanal	3	0.158 $\pm$ 0.025	56	355	0.533 $\pm$ 0.071	2	4
GS-butanal	4	0.021 $\pm$ 0.009	25	1153	0.246 $\pm$ 0.021	68	278
GS-hexenal	6	0.007 $\pm$ 0.002	16	2345	0.146 $\pm$ 0.020	74	506
GS-trans-4-hexenal	6	0.122 $\pm$ 0.075	36	297	0.078 $\pm$ 0.019	144	1858
GS-4-hydroxynonenal	9	0.005 $\pm$ 0.001	13	2600	ND	ND	ND

C_n , carbon chain length.

Table 2
AKR1B1 and AKR1B10 activity at low substrate concentrations

Data indicates the lowest substrate concentrations at which the reduction products were detected by HPLC with 50 μ l reaction mixture loaded. NA, not available; ND, no activity was detected when 0.1 – 0.5 μ M of GS-4-hydroxynonanal was used in the enzyme activity assays with 2 μ g AKR1B10.

Substrate (μ M)	1B1	1B10
Acrolein *	NA	3.00
Crotonaldehyde	40.00	0.90
Trans-2-hexenal	1.00	0.10
Trans-2, 4-hexadienal	0.80	0.05
4-Hydroxynonanal	0.60	0.10
GS-propanal	1.00	5.00
GS-butanal	0.30	0.50
GS-hexenal	0.10	0.10
GS-trans-4-hexenal	0.50	0.25
GS-4-hydroxynonanal	0.10	ND

* AKR1B10 protein for acrolein assays here was prepared without β -mercaptoethanol in buffers.