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Aldose reductase prevents aldehyde toxicity in cultured human lens epithelial cells

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Abstract

Aldehydes are widespread environmental and industrial compounds, which cause cytotoxicity, tissue damage, mutagenicity, and carcinogenicity leading to various disease conditions such as cardiovascular, bronchial, and visual complications. We have shown earlier that aldose reductase (AR) besides reducing glucose to sorbitol, efficiently reduces various toxic lipid-derived aldehydes, generated under oxidative stress, with K_m in the physiological range. We have identified the role of AR in the prevention of various lipid aldehyde-induced cytotoxic signals leading to apoptosis in human lens epithelial cells (HLEC). HLEC were cultured without or with AR inhibitors followed by addition of various saturated and unsaturated lipid aldehydes with a carbon chain length varying from C3 to C10. The cell viability was assessed by cell counts and MTT assay, and apoptosis was measured by evaluating nucleosomal degradation and caspase-3 activation using specific ELISA kits. Although all the aldehydes caused apoptosis of HLEC, the unsaturated aldehydes were more toxic than saturated aldehydes. Inhibition of AR by sorbinil potentiated while the over-expression of AR prevented the apoptosis induced by various lipid aldehydes. AR over-expression also prevented the lipid aldehyde-induced activation of caspase-3, MAPK, JNK and the expression of Bcl-2 family of proteins in HLEC. The results indicate that the lipid aldehydes generated under oxidative stress are cytotoxic to HLEC leading to apoptosis and that the reduction of lipid aldehydes by AR would prevent it.

Keywords: aldose reductase; detoxification; apoptosis; reactive oxygen species; aldehydes; lens

1. Introduction

Aldehydes are toxic compounds present in charred food, environmental contaminants such as smoke, and a host of exhausts from engines (Feron et al., 1991; Suh et al., 2000). A significant number of such aldehydes cause eye irritation and choking sensations (Krootila et al., 1986; Maurer et al., 2001; Yang et al., 2001). Toxic aldehydes are also generated in vivo when reactive oxygen species (ROS) cause oxidation of membrane polyunsaturated fatty acids (PUFAs) (Esterbauer et al., 1986; Feron et al., 1991). The peroxidation of lipids results in the generation of potentially toxic cleavage products such as aldehydes and ketones and hydroxyl-acids. Lipid aldehydes, comprise a major portion of the lipid peroxidation products, can diffuse from their site of generation and readily react with distinct molecular targets resulting in mutagenicity, genotoxicity and carcinogenicity (Uchida, 2000). In addition, lipid aldehydes have been shown to cause apoptosis of lens epithelial cells which has been correlated with cataractogenesis (Bhuyan et al., 1986; Babizhayev et al., 1988; Li et al., 1995a,b; Ansari et al., 1996; Awasthi et al., 1996; Harocopos et al., 1998; Lee et al., 2002). Further, many aldehydes have been shown to be cytotoxic, causing a loss of viability in a variety of cultured cells (Rossi et al., 1993; Townsend et al., 2001; Barrera et al., 2000; Choudhary et al., 2002). Alkenals have been shown to be more toxic than alkanals. Because of their α,β-unsaturation, alkenals are highly reactive towards cellular nucleophiles, which are important contributor to

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DNA damage, formation of DNA and protein adducts, mutagenicity and enzyme inhibition (Feron et al., 1991; Eder and Hoffman, 1993).

In order to counteract the potential lethal effects of lipid aldehydes, cells including lens epithelial, have evolved several enzyme-based detoxification systems. These are (1) aldehyde dehydrogenases (ALDH), which consist of $NAD(P)^+$-dependent enzymes that catalyze the oxidation of a wide variety of endogenously generated and exogenous aldehydes to their corresponding acids (Pietruszko et al., 1993; Vasiliou et al., 1999, 2004; Vasiliou and Nebert, 2005; Pappa et al., 2003; Sladek, 2003; Choudhary et al., 2005), (2) glutathione-*S*-transferases (GSTs), which significantly decrease aldehyde toxicity by catalyzing their conjugation with glutathione (Srivastava et al., 1994, 1996), and (3) aldo-keto reductases such as aldose reductase, which reduce aldehydes and their conjugates with glutathione to corresponding alcohols (Flynn and Green, 1993; Srivastava et al., 1999; Ramana et al., 2000).

Aldose reductase (AR) is a cytosolic, $(a/b)_8$ barrel protein with a wide tissue and species distribution (Srivastava et al., 2005), that catalyzes the first and rate limiting step of the polyol pathway of glucose metabolism i.e. reduction of glucose to sorbitol in the presence of NADPH. Both accumulation of membrane impermeable sorbitol and oxidative stress due to decrease in NADPH/NADP ratio have been implicated in tissue injury and dysfunction associated with diabetes (Sheetz and King, 2002; Chung et al., 2003). This view is supported by the observations at least in animal models, in which inhibition of AR prevents or delays several pleiotrophic complications of diabetes such as cataractogenesis, retinopathy, neuropathy and nephropathy (Srivastava et al., 2005). However, the clinical utility of AR inhibitors still remains uncertain because in several studies, inhibitors of AR did not interrupt or reverse progressive hyperglycemic injury (Sarges and Oates, 1993; Lee et al., 1995; Parry, 1999). Our recent kinetic and structural studies suggest that under normoglycemic conditions, reduction of glucose may be a secondary role of AR (Srivastava et al., 1999; Ramana et al., 2000). The purified recombinant AR displays poor affinity for glucose ($K_m = 50–100$ mM), as its active site lacks polar residues required for efficient carbohydrate binding. The preferred substrates of the enzyme are aromatic aldehydes and medium- to long-chain aliphatic aldehydes ($K_m = 30–800$ μM) derived from lipid peroxidation. We have shown that AR is an efficient catalyst for the reduction of the major toxic and abundant lipid peroxidation products such as 4-hydroxy *trans*-2-nonenal (HNE) ($K_m =$ 10–30 μM). AR inhibition is known to alter HNE metabolism in isolated perfused hearts (Srivastava et al., 1998), vascular smooth muscle cells (VSMC; Srivastava et al., 2001), lens epithelial cells (Choudhary et al., 2003), erythrocytes (Srivastava et al., 2000), and enhances HNE-induced cytotoxicity in VSMC (Ruef et al., 2000). Moreover, AR inhibitors increase the steady-state concentration of HNE and the number of apoptotic smooth muscle cells during vascular inflammation (Rittner et al., 1999). These observations suggest that reduction and detoxification of lipid-derived aldehydes may be an important function of AR particularly under normoglycemic conditions. Since lipid aldehydes have been implicated in cataractogenesis and AR inhibitors are known to prevent or delay hyperglycemic cataractogenesis and AR reduces lipid aldehyde and their conjugates with glutathione, we have investigated the effect of n-alkanals and 2-alkenals on cytotoxic signaling, leading to cell death in human lens epithelial cells (HLEC) and examined the role of AR in regulating aldehyde toxicity.

2. Materials and methods

2.1. Materials

All aldehydes were purchased from Sigma–Aldrich Chemical Co. Dulbecco's minimum essential medium (DMEM), phosphate-buffered saline (PBS), trypsin, OPTI-MEM medium and fetal bovine serum (FBS) were purchased from Gibco Laboratories (Grand Island, NY). Gentamycin was purchased from Cellgro. Sorbinil was a gift from Pfizer. Reagents used in Western blot analysis were obtained from Sigma. Antibodies against Bcl-2 family, phospho-JNK, phospho-p38 and PARP were purchased from Cell Signaling Inc. All other reagents used were of analytical grade.

2.2. Cell culture

Human lens epithelial cells were obtained from ATCC and were maintained and grown in DMEM supplemented with 10% FBS and 1% gentamycin at 37 °C in a humidified atmosphere of 5% CO_2. Cells were used between passages 6 and 11.

2.3. Measurement of cell growth

The cells were grown to confluency in indicated media, harvested by trypsinization and plated at 5000 cells/well in a 96-well plate. Cells grown at 60–80% confluency, were growth arrested for 24 h replacing fresh media containing 0.1% FBS (to prevent slow apoptosis due to complete serum starvation). After 24 h the medium was replaced with serum-free media containing either aldehydes (2 μM) alone or sorbinil (10 μM) and aldehydes (2 μM) + sorbinil for 2 h (0% serum free was maintained for 2 h to prevent conjugation of some of the aldehydes with serum proteins). After 2 h of incubation, serum was added to the media to final concentration of 0.1% and incubated for another 22 h. Aldehydes with limited solubility (pentanal, *trans*-2-hexenal and decanal) were first dissolved in dimethyl sulfoxide followed by dilution with PBS. The final concentration of dimethyl sulfoxide (DMSO) in cell culture was 0.1%. In controls respective solvents were added. Cell viability was determined by cell count by trypan blue exclusion using a hemocytometer and MTT-assay as described earlier (Ramana et al., 2002). Values from four separate experiments for each treatment were used for statistical analysis.

2.4. Transfection of human AR cDNA into HLEC

A cDNA coding for human placenta AR was subcloned into mammalian expression vector pcDNA3.1. The HLEC were transiently transfected with either human AR cDNA or an empty vector using lipofectamine 2000 transfection reagent according to manufacturer's instructions. The expression of AR protein in HLEC was verified by Western blot analysis using polyclonal antibodies against human placenta AR. Subsequently, the media was replaced with media without serum.

2.5. Apoptosis

The cells grown to confluency (60–80%) in indicated media were harvested by trypsinization and plated at 250,000 cells/well in a 6-well plate. The cell growth was arrested for 24 h by washing with PBS and then adding fresh media containing 0.1% FBS. After 24 h fresh media containing 2 μM of propanal, 2-hexenal or 2,4-nonadienal without serum incubated for another 2 h. After 2 h of incubation, serum was added to the media to make a final concentration of 0.1% and incubated for another 22 h. The apoptotic cell death was quantified using a Cell Death Detection ELISA kit (Roche Inc.) according to the manufacturer's instructions. Values from four separate experiments for each treatment were used for statistical analysis. Caspase-3 activation was measured by determining in situ cleavage of PARP protein by activated caspase-3.

2.6. Determination of caspase-3 and MAPK activation

Western blot analysis was performed by using an equal amount of protein extract (40 μg) obtained after various treatments, as indicated above. Briefly, 10% SDS–PAGE was carried out and the proteins were electroblotted to nitrocellulose filter. Specific antibodies against Bcl-2 family of proteins, phospho-MAPK and JNK were detected by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ).

2.7. Statistical analysis

Data are presented as mean ± SEM, and the P values were determined using the unpaired Student's t-test.

3. Results

3.1. Aldehydes cause inhibition of HLEC growth

To investigate the growth inhibitory effect of various aldehydes on cultured HLEC, we incubated 2 μM of saturated aldehydes ranging from carbon chain length C3 to C10, unsaturated *trans*-2-aldehydes of carbon chain length C3 to C10, and unsaturated 2,4-dienals of carbon chain length C6 to C10 in a serum-free media for 24 h as described in Section 2, and measured the cell growth by performing MTT assay and cell counting. The results shown in Fig. 1A–C suggest that all the aldehydes, irrespective of carbon chain length, caused

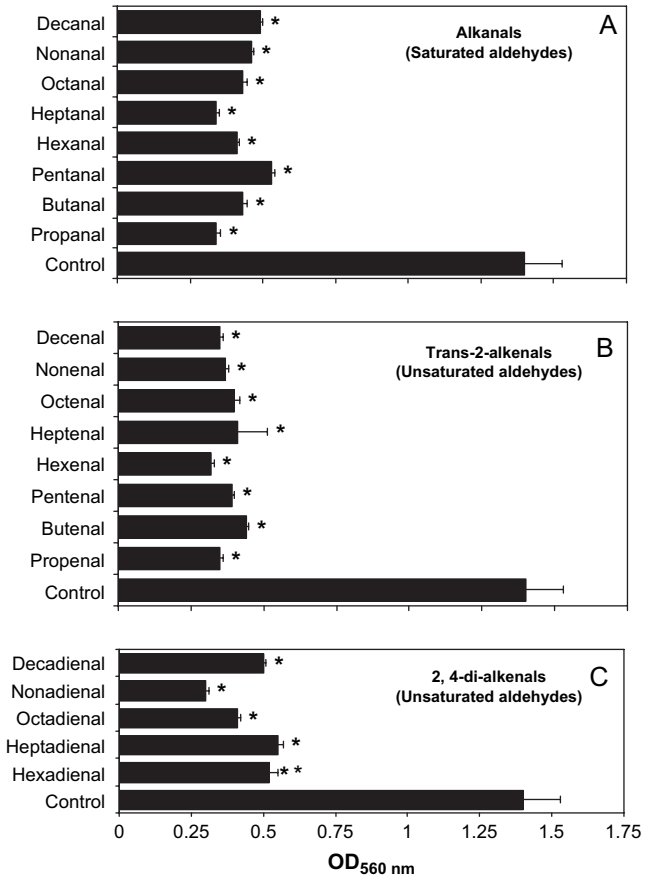

Fig. 1. Effect of saturated and unsaturated aldehydes on HLEC growth. The growth arrested HLEC (5000 cells/well in a 96-well plate) were incubated with 2 μM each of indicated (A) saturated (alkanals) aldehydes, (B) unsaturated (*trans*-2-alkenals) aldehydes or (C) unsaturated (2,4-di-alkenals) aldehydes for 24 h. The HLEC viability was determined by MTT assay as described in Section 2. The optical density (OD) was monitored at 560 nm by using Packard Spectracount™ ELISA plate reader. The data represent mean ± SEM ($n = 5$). *$P < 0.001$ as compared to control cells.

significant ($P < 0.001$) inhibition of cell growth as measured by MTT assay. Similar results were observed when we measured cell growth using cell counting (data not shown). The saturated aldehydes, propanal and heptanal caused maximum growth inhibition of HLEC followed by C6 hexanal and C4 butanal (Fig. 1A). Further, the inhibition of cell growth by C5 pentanal and C10 decanal was less than C3 propanal and others. The unsaturated aldehydes from C3 to C10 caused comparative growth inhibition. However, C8–C10 alkenals were more apoptotic than C8–C10 alkanals. Among the *trans*-2-alkenals, C6 hexenal caused maximum growth inhibition followed by C3 propenal and others. The C5 pentenal was more apoptotic than C5 pentanal (Fig. 1B). 2,4-Dialkenals were also apoptotic and the growth inhibition was not more than that caused by *trans*-2-alkenals (Fig. 1C). Nonadienal caused maximum growth inhibition amongst all the aldehydes studied. These results suggest that *trans*-2-aldehydes are more toxic than alkanals and 2,4-dialkenals in inhibiting HLEC growth. We have also investigated the effect of increasing concentration of these aldehydes on HLEC apoptosis. Our results show that at <2 μM concentrations only 30–40% apoptosis

was observed while at >2 μM concentrations up to100% apoptosis and necrosis was observed (data not shown). Therefore we have used 2 μM aldehydes, which cause ~60% apoptosis, throughout the study.

3.2. AR inhibition exacerbates aldehyde-induced inhibition of HLEC growth

We have shown earlier that aldehydes are excellent substrates (K_m in μM range) of AR, suggesting that this enzyme may be involved in the detoxification of toxic aldehydes (Srivastava et al., 1999). To examine this, we incubated HLEC with AR inhibitor sorbinil or the solvent followed by incubation with 2 μM each of the aldehydes, propanal, hexenal and 2,4-nonadienal and measured the cell growth by cell counting and MTT assay. All the aldehydes studied caused significant decrease in HLEC growth and AR inhibition exacerbated the aldehyde effect (Fig. 2A,B), suggesting the requirement of AR for the disposition of aldehydes and their toxic effects in HLEC.

3.3. AR over-expression prevents aldehyde-induced inhibition of HLEC growth

Since AR inhibitors worsen the HLEC growth inhibition caused by aldehydes, we next examined if AR over-expression would prevent the aldehyde-induced growth inhibition in HLEC. The HLEC were transiently transfected with human placenta AR-cDNA vector that caused over-expression of AR protein by more than 25 fold (Fig. 3B, inset). The untransfected, vector and AR-cDNA vector-transfected HLEC were incubated with 2 μM each of propanal, 2-hexenal and 2,4-nonadienal for 24 h and cell growth was determined by cell counting as well as MTT assay. The results shown in Fig. 3A and B suggest that the aldehydes in untransfected or vector alone transfected cells significantly inhibited HLEC growth but cells over-expressing AR showed remarkable resistance against aldehydes-induced inhibition of cell growth, suggesting that AR prevents aldehyde-induced cell death in HLEC.

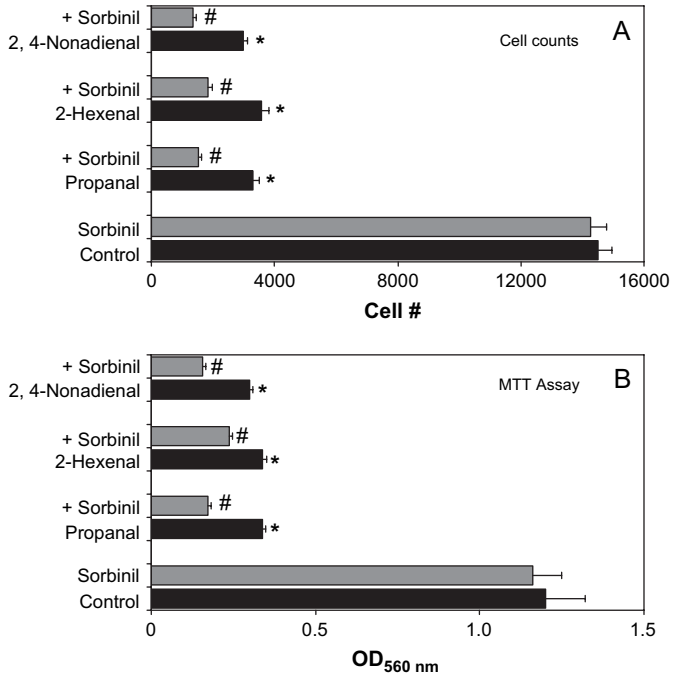

Fig. 2. Inhibition of aldose reductase promotes aldehyde-induced HLEC death. The growth arrested HLEC (5000 cells/well in a 96-well plate) were incubated with 2 μM each of propanal, 2-hexenal or 2,4-nonadienal in the absence and presence of AR inhibitor sorbinil (10 μM) for 24 h. The HLEC growth was determined by (A) measuring cell counts and by (B) MTT assay, as described in Section 2. The cell counting was carried out by using hemocytometer and the optical density (OD) was monitored at 560 nm by using Packard Spectracount ELISA plate reader. The data represent mean ± SEM ($n = 5$). $^{\#}$,$^{*}P < 0.001$ as compared to control cells.

3.4. AR over-expression prevents aldehyde-induced apoptosis of HLEC

To examine if the growth inhibition of HLEC by aldehydes and protection by AR reflects apoptotic cell death, we measured apoptosis by using Roche's Cell Death ELISA kit, which measures nucleosomal degradation. The results shown in Fig. 4 suggest that propanal, hexenal and nonadienal caused

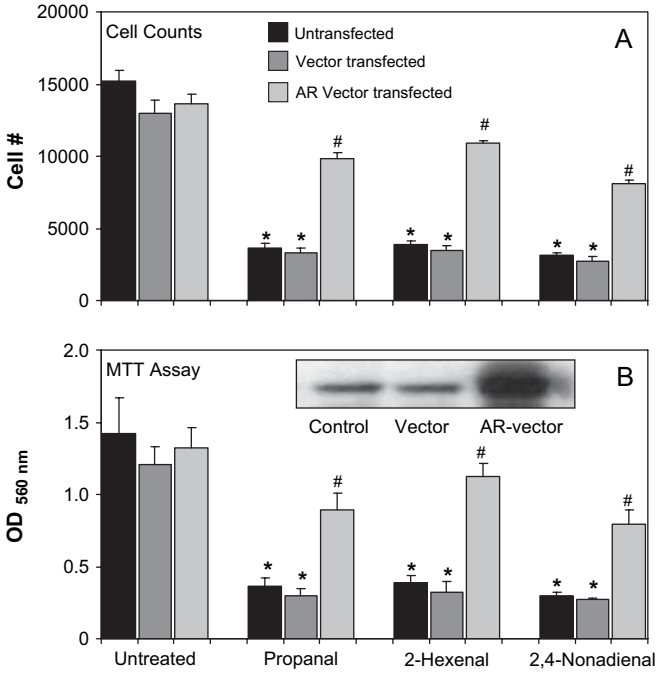

Fig. 3. Over-expression of AR inhibits aldehyde-induced HLEC death. The HLEC were transiently transfected with pCDNA 3.1 vector containing human placenta AR cDNA or vector alone. The transfected and untransfected HLEC were growth arrested for 24 h, followed by incubation with 2 μM each of propanal, 2-hexenal or 2,4-nonadienal for an additional 24 h. The HLEC growth was determined by (A) measuring cell counts and by (B) MTT assay, as described in Section 2. The cell counting was carried out by using hemocytometer and the optical density (OD) was monitored at 560 nm by using Packard Spectracount ELISA plate reader. The data represent mean ± SEM ($n = 5$). $^{*}P < 0.001$ as compared to control cells, $^{\#}P < 0.001$ as compared to vector alone transfected cells. The inset in (B) shows the AR protein levels in untransfected and transfected HLEC.

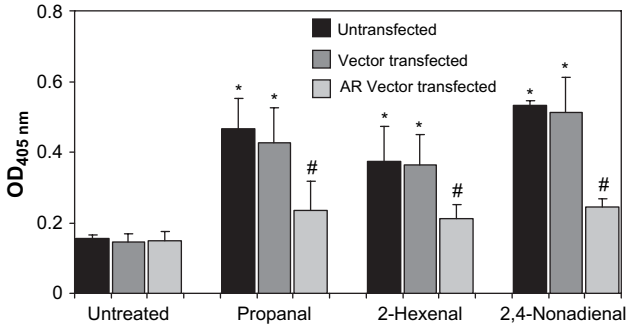

Fig. 4. Over-expression of AR inhibits aldehyde-induced HLEC apoptosis. The HLEC were transiently transfected with pCDNA 3.1 vector containing human placenta AR cDNA or vector alone. The transfected and untransfected HLEC were growth arrested for 24 h, followed by incubation with 2 μM each of propanal, 2-hexenal or 2,4-nonadienal for an additional 24 h. The HLEC apoptosis was determined by measuring nucleosomal degradation using a Cell Death detection ELISA kit (Roche). The optical density (OD) was monitored at 405 nm using a Packard Spectracount ELISA plate reader. The data represent mean ± SEM ($n = 5$). $^{*}P < 0.001$ as compared to control cells, $^{\#}P < 0.001$ as compared to vector alone transfected cells.

apoptosis of HLEC. As compared to propanal and 2-hexenal, 2,4-nonadienal showed greater nucleosomal degradation. AR over-expressing cells were significantly protected from aldehyde-induced apoptosis.

3.5. AR over-expression prevents aldehyde -induced regulation of Bcl-2 family of proteins

In order to investigate if AR over-expression prevents aldehyde toxicity, we examined the effect of aldehydes on the expression of pro-apoptotic and anti-apoptotic Bcl-2 family of proteins in normal and AR over-expressing HLEC. In vector alone transfected cells stimulation with propanal, 2-hexenal and 2,4-nonadienal down-regulated the expression of anti-apoptotic proteins, Bcl-2 and Bcl-xl and up-regulated the expression of pro-apoptotic proteins Bax and Bad (Fig. 5). As compared to propanal, 2,4-nonadienal followed by 2-hexenal caused increased expression of Bad. However, no significant difference was observed in the expression of Bax by all the three aldehydes. In 2-hexenal-treated cells, decrease in the Bcl-2 levels was significantly more as compared to propanal and 2,4-nonadienal. Overexpression of AR completely prevented the down-regulation of Bcl-2 and Bcl-xl and up-regulation of Bax and Bad by all the three aldehydes. There was no significant difference in the levels of these proteins among the controls. These observations indicate that overexpression of AR by itself does not affect the expression of Bcl-2 proteins, but it abolishes aldehyde-induced HLEC activation by interrupting aldehyde signaling.

3.6. AR over-expression prevents aldehyde-induced activation of Caspase-3, p38-MAPK and JNK

To examine the effects of AR inhibition on known signaling mediators of apoptosis, we measured aldehyde-induced caspase-3 activation and phosphorylation of p38-MAPK and JNK in HLEC. As shown in Fig. 6A, stimulation of HLEC with

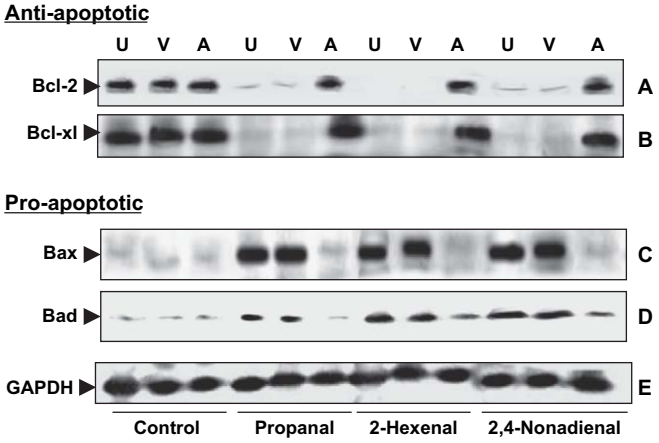

Fig. 5. Over-expression of AR inhibits aldehyde-induced regulation of Bcl-2 family of proteins. The HLEC were transiently transfected with pCDNA 3.1 vector containing human placenta AR cDNA or vector alone. The transfected and untransfected HLEC were growth arrested for 24 h, followed by incubation with 2 μM each of propanal, 2-hexenal or 2,4-nonadienal for additional 24 h. Western blot analysis was carried out to determine the levels of indicated Bcl-2 family proteins by using specific antibodies against (A) Bcl-2, (B) Bcl-xl, (C) Bax and (D) Bad, as described in Section 2. Western blot analysis was carried out at least three times and the figures shown are representative of three independent analyses. (E) Western blot analysis with anti-GAPDH antibodies to demonstrate equal loading of protein in all the cases.

propanal, 2-hexenal and 2,4-nonadienal caused activation ofcaspase-3 as determined by PARP cleavage. However, when HLEC over-expressing AR were treated with these three aldehydes, no PARP cleavage was observed, suggesting that over-expression of AR provides resistance to aldehyde-mediated activation of caspase-3. To determine how over-expression of AR prevents aldehyde signaling leading to apoptosis, we tested the effect of AR over-expression on aldehyde-induced activation of JNK and p38 MAPK. As shown in Fig. 6B and C, there was a marked increase in the phosphorylated forms of JNK and p38 MAPK in the presence of the aldehydes. AR over-expression significantly attenuated aldehyde-induced JNK and p38

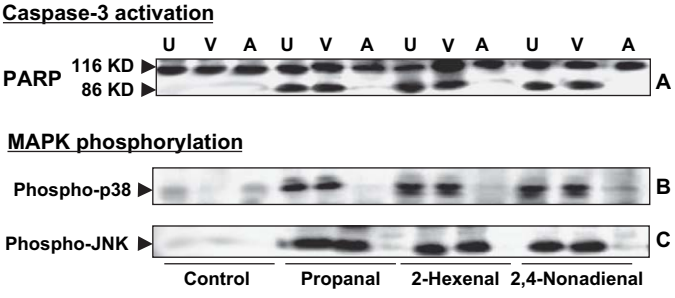

Fig. 6. Over-expression of AR inhibits aldehyde-induced activation of caspase-3 and MAP-kinases. The HLEC were transiently transfected with pCDNA 3.1 vector containing human placenta AR cDNA or vector alone. The transfected and untransfected HLEC were growth arrested for 24 h, followed by incubation with 2 μM each of propanal, 2-hexenal or 2,4-nonadienal for an additional 24 h. Western blot analysis was carried out to determine activation of caspase-3 by PARP cleavage and phosphorylation of p38 MAPK and JNK by using specific antibodies against (A) PARP, (B) phospho-p38 and (C) phospho-JNK, as described in Section 2. Western blot analysis was carried out at least three times and the figures shown are representative of three independent analyses.

phosphorylation, suggesting that AR mediates the cytotoxic signals of aldehydes that cause apoptosis of HLEC.

4. Discussion

Ocular tissues are constantly exposed to reactive oxygen species (ROS), originating from intrinsic and extrinsic sources. Environmental contaminants such as smoke and vehicle exhausts cause severe eye irritation and therefore their efficient detoxification is required to ensure the normal functioning of the ocular tissues (Krootila et al., 1986; Maurer et al., 2001; Yang et al., 2001). It has been reported that thermal stressing of culinary oils and fats according to standard frying practices (domestic or otherwise) gives rise to oxygen radical-mediated autoxidation of PUFAs which in turn produce aldehydes via a process that involves the scission of preformed alkoxyl radicals (Sheerin et al., 1997). The concentrations of such PUFA-derived autoxidation generated aldehydic species (*n*-alkanals, *trans*-2-alkenals, *trans*,*trans*- and *cis*,*trans*-alka-2,4-dienals, and 4-hydroxy-*trans*-2-alkenals) have the capacity to exert a variety of toxicological effects by exhibiting extremely high reactivity with critical biomolecules such as DNA base adducts, proteins such as low-density lipoprotein, peptides, free amino acids, and endogenous thiols such as glutathione (Uchida, 2000). Indeed, a wealth of information concerning the generation and toxicity of these agents as terminal products of in vivo lipid peroxidation processes is currently available in the literature (Meagher and FitzGerald, 2000; Marnett, 2002; Yang et al., 2003; Bartsch and Nair, 2004; Petersen and Doorn, 2004).

Aldose reductase is the first and rate-limiting enzyme of the polyol pathway of glucose metabolism that reduces glucose to sorbitol and is implicated in the etiology of secondary diabetic complications (Srivastava et al., 2005). During hyperglycemia, >30% of glucose is metabolized by the sorbitol pathway, whereas in normoglycemic conditions <3% of glucose is metabolized by this pathway (Gonzalez et al., 1984). However, the physiological function(s) of this pathway is not clearly known in normoglycemic conditions. In the testis, it is responsible for the production of fructose, a major energy source for sperms (Kobayashi et al., 2002; Frenette et al., 2004). In the kidney, it is involved in osmoregulation, a concomitant increase in sorbitol, an osmolyte, can induce the expression of various stress response genes (Garcia-Perez and Burg, 1991). We have shown it earlier that due to hydrophobicity of the active site glucose is not the preferred substrate of AR ($K_m = 50-100$ mM), rather this enzyme is more effective in reducing a large variety of aromatic and aliphatic aldehydes and their conjugates with glutathione (Srivastava et al., 1999; Ramana et al., 2000). We and others have shown that AR can reduce methylglyoxal ($K_m = 7.8$ μM), 4-hydroxynonenal ($K_m = 8-30$ μM), acrolein ($K_m = 0.8$ mM) and other saturated and unsaturated aldehydes ($K_m =$ μM range) (Srivastava et al., 1995; Vander Jagt et al., 1995, 2001). Our kinetic studies also demonstrate that AR efficiently catalyzes the reduction of GS-aldehydes more efficiently (10- to 100-fold) than their parent aldehydes because the active site of AR has GSH-binding site (Dixit et al., 2000; Ramana et al., 2000). Thus, the main function of AR in the cells during normoglycemic conditions may be detoxification of various cytotoxic aldehydes. Besides AR, ALDH1A1, which detoxifies HNE by oxidation in the presence of NAD to the corresponding acid, has been shown to prevent ROS toxicity in the human and rat lens epithelial cells (Choudhary et al., 2003, 2005).

The normal physiological concentrations of aldehydes such as HNE in the plasma and erythrocytes of humans and experimental animals have been reported to be 0.1–10 μM, but may increase by several fold, upon exposure to various oxidants, imbalance of physiological antioxidants with oxidants, bacterial infections initiated and ROS-induced lipid peroxidation (Selley, 1997; Awasthi et al., 2004). In bio-membranes, the concentration of HNE is shown to be between 10 μM and 1.0 mM following oxidative stress (Esterbauer and Ramos, 1996). Increased concentration of free HNE and HNE-modified proteins is well documented under several pathological conditions, including autoimmune diseases, renal failure, Alzheimer's disease, Parkinson's disease, alcoholic liver disease, and in infants with chronic lung disease and atherosclerosis (Uchida et al., 1994; Yoritaka et al., 1996; Grune et al., 1997; Ogihara et al., 1999; Picklo et al., 2002). We have shown earlier that a major portion of HNE metabolism in erythrocytes and various cell lines such as VSMC and lens epithelial cells is via conjugation with glutathione, which can be transported out of the cells (Choudhary et al., 2003; Srivastava et al., 2003). We and others have previously shown that HNE and other lipid peroxidation-derived α,β-unsaturated aldehydes can form Michael adducts with GSH spontaneously or catalyzed by glutathione-*S*-transferase (Srivastava et al., 1995; Vilardo et al., 2001). Our earlier studies also show that the lipid peroxidation-derived aldehydes and their glutathione conjugates are efficiently reduced by the aldose reductase (Ramana et al., 2000). Further, our molecular modeling studies also suggest that the active site of aldose reductase can accommodate HNE and structurally related lipid peroxidation-derived aldehydes as well as their glutathione conjugates (Srivastava et al., 1999). A prominent role of aldose reductase in reducing the glutathione conjugates of HNE is indicated by the observation that the formation of GS-DHN was significantly inhibited in rat erythrocytes by two, structurally unrelated, aldose reductase inhibitors, sorbinil and tolrestat (Srivastava et al., 2000). The aldose reductase-mediated catalysis is protective since, by reducing the aldehydes generated by ROS-induced lipid peroxidation, the enzyme may prevent the spontaneous dissociation of GS-aldehydes, which could deliver the aldehyde to non-exposed sites causing transcellular and transorgan toxicity. Thus, the regulation of AR expression would play an important role in ROS-mediated cytotoxicity. Recent studies have shown the presence of binding sites for redox-regulated transcription factors such as AP-1 and NF-κB in the promoter of the AR gene (Iwata et al., 1999). This suggests that AR may be a significant component of antioxidant defenses involved in redox cell signaling. AR is also induced by growth factors, tumor necrosis factor

(TNF)-α, and interferon, all known to generate ROS (Srivastava et al., 2005). Expression of AR is also enhanced in T cells, macrophages, liver, lens and VSMC under high oxidative stress that results in increased HNE formation, suggesting redox regulation of the AR gene in several tissues (Jedziniak et al., 1981; Rittner et al., 1999; Ramana et al., 2004a,b; Brown et al., 2005). Given the extensive evidence implicating ROS as mediators of cell growth and differentiation (Srivastava et al., 2005), it appears likely that cytokine and growth factor-induced up-regulation of AR is due to ROS generated during signaling. The association of ROS and AR is supported by the observations that AR inhibitors attenuate glucose-induced oxidative, nitrosative stress and superoxide production in retinal pericytes, bovine aortic endothelial cells and rabbit aorta (Tesfamariam et al., 1993; Dagher et al., 2004; Obrosova et al., 2005; Ramana et al., 2005). The strongest evidence that AR is involved in mediating growth comes from our studies showing that inhibition of AR prevents proliferation of cultured VSMC in response to fibroblast growth factor (FGF), TNF-α and thrombin (Ramana et al., 2002). In addition, the observation that the AR inhibitor epalrestat prevents initial thickening in coronary arteries of galactose-fed beagle dogs provides additional support for the role of AR in abnormal VSMC growth (Kasuya et al., 1999). In contrast, in rat hepatoma cell lines inhibition of aldose reductase decreases 3-deoxyglucosone and glyceraldehyde-induced viability of the cells (Takahashi et al., 1995). Similarly, AR inhibition also enhanced apoptosis induced by hydrogen peroxide and HNE in A7r5 cells (Spycher et al., 1997), and daunorubicin in HepG2 cells (Lee et al., 2001), presumably by preventing AR from detoxifying these aldehydes responsible for cytotoxicity. On the other hand, oxidative stress that generates lipid aldehydes is known to be essential component of cytokine, chemokine, growth factors, and endotoxin signals that cause NF-κB and AP1, activation followed by cellular toxicity (Ramana et al., 2002, 2003, 2004b). We have indeed shown earlier that reduced lipid aldehydes and/or their glutathione conjugates are major mediators of NF-κB activation (Srivastava et al., 2005). Inhibition or ablation of AR prevented the TNF-α, high glucose and endotoxin-induced NF-κB activation in VSMC and vascular endothelial cells (Ramana et al., 2003, 2004a,b). Similarly, AR could have a dual function in HLEC: (1) protection against the cytotoxic effects of aldehydes by reducing them to corresponding alcohol and (2) mediation of cytokines, chemokines and growth factors-induced NF-κB activation and cell growth. Further studies are required to understand the therapeutic doses and harmful/beneficial effects of long-term use of AR inhibitors.

In summary, we have shown that over-expression of AR in HLEC provides protection against cytotoxic signals by aldehydes. Decreased cytotoxicity of aldehydes in AR-overexpressing cells appears to be due to attenuation of apoptotic signaling, inhibition of stress-activated kinases (JNK and p38), and diminished stimulation of caspase-3. Significantly, over-expression of AR prevented the aldehyde -induced decrease in the abundance of Bcl-2, Bcl-xl and up-regulation of Bax and Bad. Our results thus indicate that the cytotoxic signals of aldehydes that cause HLEC apoptosis are mediated by AR, possibly by regulating the reduction of lipid aldehydes and their glutathione conjugates.

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References

Ansari, N.H., Wang, L., Srivastava, S.K., 1996. Role of lipid aldehydes in cataractogesis: 4-hydroxynonenal -induced cataract. Biochem. Mol. Med. 58, 25–30.

Awasthi, S., Srivatava, S.K., Piper, J.T., Singhal, S.S., Chaubey, M., Awasthi, Y.C., 1996. Curcumin protects against 4-hydroxy-2-nonenal-induced cataract formation in rat lenses. Am. J. Clin. Nutr. 64, 761–766.

Awasthi, Y.C., Yang, Y., Tiwari, N., Patrick, B., Sharma, A., Li, J., Awasthi, S., 2004. Regulation of 4-hydroxynonenal-mediated signaling by glutathione S-transferases. Free Radic. Biol. Med. 37, 607–619.

Babizhayev, M.A., Deyev, A.I., Linberg, L.F., 1988. Lipid peroxidation as a possible cause of cataract. Mech. Aging Dev. 44, 69–89.

Barrera, G., Pizzimenti, S., Serra, A., Fazio, V.M., Canuto, R.A., Dianzani, M.U., 2000. Effect of bioactive aldehydes on cell proliferation and c-myc expression in HL-60 human leukemic cells. Cancer Detect. Prev. 24, 244–251.

Bartsch, H., Nair, J., 2004. Oxidative stress and lipid peroxidation-derived DNA-lesions in inflammation driven carcinogenesis. Cancer Detect. Prev. 28, 385–391.

Bhuyan, K.C., Master, R.W., Coles, R.S., Bhuyan, D.K., 1986. Molecular mechanisms of cataractogenesis: IV. Evidence of phospholipid. malondialdehyde adduct in human senile cataract. Mech. Ageing Dev. 34, 289–296.

Brown, K.E., Broadhurst, K.A., Mathahs, M.M., Kladney, R.D., Fimmel, C.J., Srivastava, S.K., Brunt, E.M., 2005. Immunodetection of aldose reductase in normal and diseased human liver. Histol. Histopathol. 20, 429–436.

Choudhary, S., Zhang, W., Zhou, F., Campbell, G.A., Chan, L.L., Thompson, E.B., Ansari, N.H., 2002. Cellular lipid peroxidation end-products induce apoptosis in human lens epithelial cells. Free Radic. Biol. Med. 32, 360–369.

Choudhary, S., Srivastava, S., Xiao, T., Andley, U.P., Srivastava, S.K., Ansari, N.H., 2003. Metabolism of lipid derived aldehyde, 4-hydroxynonenal in human lens epithelial cells and rat lens. Invest. Ophthalmol. Vis. Sci. 44, 2675–2682.

Choudhary, S., Xiao, T., Srivastava, S., Zhang, W., Chan, L.L., Vergara, L.A., Van Kuijk, F.J., Ansari, N.H., 2005. Toxicity and detoxification of lipid-derived aldehydes in cultured retinal pigmented epithelial cells. Toxicol. Appl. Pharmacol. 204, 122–134.

Chung, S.S., Ho, E.C., Lam, K.S., Chung, S.K., 2003. Contribution of polyol pathway to diabetes-induced oxidative stress. J. Am. Soc. Nephrol. 14, S233–S236.

Dagher, Z., Park, Y.S., Asnaghi, V., Hoehn, T., Gerhardinger, C., Lorenzi, M., 2004. Studies of rat and human retinas predict a role for the polyol pathway in human diabetic retinopathy. Diabetes 53, 2404–2411.

Dixit, B.L., Balendiran, G.K., Watowich, S.J., Srivastava, S., Ramana, K.V., Petrash, J.M., Bhatnagar, A., Srivastava, S.K., 2000. Kinetic and structural characterization of the glutathione-binding site of aldose reductase. J. Biol. Chem. 275, 21587–21595.

Eder, E., Hoffman, C., 1993. Identification and characterization of deoxyguanosine adducts of mutagenic beta-alkyl-substituted acrolein congeners. Chem. Res. Toxicol. 6, 486–494.

Esterbauer, E., Ramos, P., 1996. Chemistry and pathophysiology of oxidation of LDL. Rev. Physiol. Biochem. Pharmacol. 127, 31–54.

Esterbauer, H., Benedetti, A., Lang, J., Fulceri, R., Fauler, G., Comporti, M., 1986. Studies on the mechanism of formation of 4-hydroxynonenal during microsomal lipid peroxidation. Biochim. Biophys. Acta. 876, 154–166.

Feron, V.J., Til, H.P., de Vrijer, F., Woutersen, R.A., Cassee, F.R., van Bladeren, P.J., 1991. Aldehydes: occurrence, carcinogenic potential, mechanism of action and risk assessment. Mutat. Res. 259, 363–385.

Flynn, T.G., Green, N.C., 1993. The aldo-keto reductases: an overview. Adv. Exp. Med. Biol. 328, 251–257.

Frenette, G., Lessard, C., Sullivan, R., 2004. Polyol pathway along the bovine epididymis. Mol. Reprod. Dev. 69, 448–456.

Garcia-Perez, A., Burg, M.B., 1991. Renal medullary organic osmolytes. Physiol. Rev. 71, 1081–1115.

Gonzalez, R.G., Barnett, P., Aguayo, J., Cheng, H.M., Chylack, L.T., 1984. Direct measurement of polyol pathway activity in the ocular lens. Diabetes 33, 196–199.

Grune, T., Michel, P., Sitte, N., Eggert, W., Albrecht-Nebe, H., Esterbauer, H., Siems, W.G., 1997. Increased levels of 4-hydroxynonenal modified proteins in plasma of children with autoimmune diseases. Free Radic. Biol. Med. 23, 357–360.

Harocopos, G.J., Alvares, K.M., Kolker, A.E., Beebe, D.C., 1998. Human age-related cataract and lens epithelial cell death. Invest. Ophthalmol. Vis. Sci. 39, 2696–2706.

Iwata, T., Sato, S., Jimenez, J., McGowan, M., Moroni, M., Dey, A., Ibaraki, N., Reddy, V.N., Carper, D., 1999. Osmotic response element is required for the induction of aldose reductase by tumor necrosis factor-alpha. J. Biol. Chem. 274, 7993–8001.

Jedziniak, J.A., Chylack Jr., L.T., Cheng, H.M., Gillis, M.K., Kalustian, A.A., Tung, W.H., 1981. The sorbitol pathway in the human lens: aldose reductase and polyol dehydrogenase. Invest. Ophthalmol. Vis. Sci. 20, 314–326.

Kasuya, Y., Ito, M., Nakamura, J., Hamada, Y., Nakayama, M., Chaya, S., Komori, T., Naruse, K., Nakashima, E., Kato, K., Koh, N., Hotta, N., 1999. An aldose reductase inhibitor prevents the intimal thickening in coronary arteries of galactose-fed beagle dogs. Diabetologia 42, 1404–1409.

Kobayashi, T., Kaneko, T., Iuchi, Y., Matsuki, S., Takahashi, M., Sasagawa, I., Nakada, T., Fujii, J., 2002. Localization and physiological implication of aldose reductase and sorbitol dehydrogenase in reproductive tracts and spermatozoa of male rats. J. Androl. 23, 674–683.

Krootila, K., Uusitalo, H., Lehtosalo, J.I., Palkama, A., 1986. Effect of topical chemical irritation on the blood-aqueous barrier of the rat eye. Ophthalmic Res. 18, 248–252.

Lee, A.Y., Chung, S.K., Chung, S.S., 1995. Demonstration that polyol accumulation is responsible for diabetic cataract by the use of transgenic mice expressing the aldose reductase gene in the lens. Proc. Natl. Acad. Sci. USA 92, 2780–2784.

Lee, K.W., Ko, B.C., Jiang, Z., Cao, D., Chung, S.S., 2001. Overexpression of aldose reductase in liver cancers may contribute to drug resistance. Anticancer Drugs 12, 129–132.

Lee, E.H., Wan, X.H., Song, J., Kang, J.J., Cho, J.W., Seo, K.Y., Lee, J.H., 2002. Lens epithelial cell death and reduction of anti-apoptotic protein Bcl-2 in human anterior polar cataracts. Mol. Vis. 8, 235–240.

Li, W.C., Kuszak, J.R., Dunn, K., Wang, R.R., Ma, W., Wang, G.M., Spector, A., Leib, M., Cotliar, A.M., Weiss, M., et al., 1995a. Lens epithelial cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals. J. Cell Biol. 130, 169–181.

Li, W.C., Kuszak, J.R., Wang, G.M., Wu, Z.Q., Spector, A., 1995b. Calcimycin-induced lens epithelial cell apoptosis contributes to cataract formation. Exp. Eye Res. 61, 91–98.

Marnett, L.J., 2002. Oxy radicals, lipid peroxidation and DNA damage. Toxicology 181-182, 219–222.

Maurer, J.K., Molai, A., Parker, R.D., Li, L.I., Carr, G.J., Petroll, W.M., Cavanagh, H.D., Jester, J.V., 2001. Pathology of ocular irritation with acetone, cyclohexanol, parafluoroaniline, and formaldehyde in the rabbit low-volume eye test. Toxicol. Pathol. 29, 187–199.

Meagher, E.A., FitzGerald, G.A., 2000. Indices of lipid peroxidation in vivo: strengths and limitations. Free Radic. Biol. Med. 28, 1745–1750.

Obrosova, I.G., Pacher, P., Szabo, C., Zsengeller, Z., Hirooka, H., Stevens, M.J., Yorek, M.A., 2005. Aldose reductase inhibition counteracts oxidative-nitrosative stress and poly(ADP-ribose) polymerase activation in tissue sites for diabetes complications. Diabetes 54, 234–242.

Ogihara, T., Hirano, K., Morinobu, T., Kim, H.S., Hiroi, M., Ogihara, H., Tamai, H., 1999. Raised concentrations of aldehyde lipid peroxidation products in premature infants with chronic lung disease. Arch. Dis. Child. (Fetal Neonatal Ed.) 80, F21–F25.

Pappa, A., Estey, T., Manzer, R., Brown, D., Vasiliou, V., 2003. Human aldehyde dehydrogenase 3A1 (ALDH3A1): biochemical characterization and immunohistochemical localization in the cornea. Biochem. J. 376, 615–623.

Parry, G.J., 1999. Management of diabetic neuropathy. Am. J. Med. 107, 27S–33S.

Petersen, D.R., Doorn, J.A., 2004. Reactions of 4-hydroxynonenal with proteins and cellular targets. Free Radic. Biol. Med. 37, 937–945.

Picklo, M.J., Montine, T.J., Amarnath, V., Neely, M.D., 2002. Carbonyl toxicology and Alzheimer's disease. Toxicol. Appl. Pharmacol. 184, 187–197.

Pietruszko, R., Abriola, D.P., Blatter, E.E., Mukerjee, N., 1993. Aldehyde dehydrogenase: aldehyde dehydrogenation and ester hydrolysis. Adv. Exp. Med. Biol. 328, 221–231.

Ramana, K.V., Dixit, B.L., Srivastava, S., Balendiran, G.K., Srivastava, S.K., Bhatnagar, A., 2000. Selective recognition of glutathiolated aldehydes by aldose reductase. Biochemistry 39, 12172–12180.

Ramana, K.V., Chandra, D., Srivastava, S., Bhatnagar, A., Aggarwal, B.B., Srivastava, S.K., 2002. Aldose reductase mediates mitogenic signaling in vascular smooth muscle cells. J. Biol. Chem. 277, 32063–32070.

Ramana, K.V., Friedrich, B., Bhatnagar, A., Srivastava, S.K., 2003. Aldose reductase mediates cytotoxic signals of hyperglycemia and TNF-alpha in human lens epithelial cells. FASEB J. 17, 315–317.

Ramana, K.V., Friedrich, B., Srivastava, S., Bhatnagar, A., Srivastava, S.K., 2004a. Activation of nuclear factor-kappaB by hyperglycemia in vascular smooth muscle cells is regulated by aldose reductase. Diabetes 53, 2910–2920.

Ramana, K.V., Bhatnagar, A., Srivastava, S.K., 2004b. Inhibition of aldose reductase attenuates TNF-alpha-induced expression of adhesion molecules in endothelial cells. FASEB J. 18, 1209–1218.

Ramana, K.V., Friedrich, B., Tammali, R., West, M.B., Bhatnagar, A., Srivastava, S.K., 2005. Requirement of aldose reductase for the hyperglycemic activation of protein kinase C and formation of diacylglycerol in vascular smooth muscle cells. Diabetes 54, 818–829.

Rittner, H.L., Hafner, V., Klimiuk, P.A., Szweda, L.I., Goronzy, J.J., Weyand, C.M., 1999. Aldose reductase functions as a detoxification system for lipid peroxidation products in vasculitis. J. Clin. Invest. 103, 1007–1013.

Rossi, M.A., Fidale, F., Di Mauro, C., Esterbauer, H., Dianzani, M.U., 1993. Effect of 4-hydroxy-2,3-trans-nonenal and related aldehydes on phospholipase C activity of rat neutrophils. Int. J. Tissue React. 15, 201–205.

Ruef, J., Liu, S.Q., Bode, C., Tocchi, M., Srivastava, S., Runge, M.S., Bhatnagar, A., 2000. Involvement of aldose reductase in vascular smooth muscle cell growth and lesion formation after arterial injury. Arterioscler Thromb. Vasc. Biol. 20, 1745–1752.

Sarges, R., Oates, P.J., 1993. Aldose reductase inhibitors: recent developments. Prog. Drug Res. 40, 99–161.

Selley, M.L., 1997. Determination of the lipid peroxidation product (E)-4-hydroxy-2-nonenal in clinical samples by gas chromatography-negative-ion chemical ionization mass spectrometry of the O-pentafluorobenzyl oxime. J. Chromatogr. B. (Biomed. Sci. Appl.) 691, 263–268.

Sheerin, A.N., Silwood, C., Lynch, E., Grootveld, M., 1997. Production of lipid peroxidation products in culinary oils and facts during episodes of thermal stressing: a high field 1H NMR investigation. Biochem. Soc. Trans. 25, 495S.

Sheetz, M.J., King, G.L., 2002. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. J. Am. Med. Assoc. 288, 2579–2588.

Sladek, N.E., 2003. Human aldehyde dehydrogenases: potential pathological, pharmacological, and toxicological impact. J. Biochem. Mol. Toxicol 17, 7–23.

Spycher, S.E., Tabataba-Vakili, S., O'Donnell, V.B., Palomba, L., Azzi, A., 1997. Aldose reductase induction: a novel response to oxidative stress of smooth muscle cells. FASEB J. 11, 181–188.

Srivastava, S.K., Singhal, S.S., Bajpai, K.K., Chaubey, M., Ansari, N.H., Awasthi, Y.C., 1994. A group of novel glutathione S-transferase isozymes showing high activity towards 4-hydroxy-2-nonenal are present in bovine ocular tissues. Exp. Eye Res. 59, 151–159.

Srivastava, S., Chandra, A., Bhatnagar, A., Srivastava, S.K., Ansari, N.H., 1995. Lipid peroxidation product, 4-hydroxynonenal and its conjugate with GSH are excellent substrates of bovine lens aldose reductase. Biochem. Biophys. Res. Commun. 217, 741–746.

Srivastava, S.K., Singhal, S.S., Awasthi, S., Pikula, S., Ansari, N.H., Awasthi, Y.C., 1996. A glutathione S-transferases isozyme (bGST 5.8) involved in the metabolism of 4-hydroxy-2-trans-nonenal is localized in bovine lens epithelium. Exp. Eye Res. 63, 329–337.

Srivastava, S., Chandra, A., Wang, L.F., Seifert Jr., W.E., DaGue, B.B., Ansari, N.H., Srivastava, S.K., Bhatnagar, A., 1998. Metabolism of the lipid peroxidation product, 4-hydroxy-trans-2-nonenal, in isolated perfused rat heart. J. Biol. Chem. 273, 10893–10900.

Srivastava, S., Watowich, S.J., Petrash, J.M., Srivastava, S.K., Bhatnagar, A., 1999. Structural and kinetic determinants of aldehyde reduction by aldose reductase. Biochemistry 38, 42–54.

Srivastava, S., Dixit, B.L., Cai, J., Sharma, S., Hurst, H.E., Bhatnagar, A., Srivastava, S.K., 2000. Metabolism of lipid peroxidation product, 4-hydroxynonenal (HNE) in rat erythrocytes: role of aldose reductase. Free Radic. Biol. Med. 29, 642–651.

Srivastava, S., Conklin, D.J., Liu, S.Q., Prakash, N., Boor, P.J., Srivastava, S.K., Bhatnagar, A., 2001. Identification of biochemical pathways for the metabolism of oxidized low-density lipoprotein derived aldehyde-4-hydroxy trans-2-nonenal in vascular smooth muscle cells. Atherosclerosis 158, 339–350.

Srivastava, S.K., Ramana, K.V., Bhatnagar, A., 2005. Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options. Endocr. Rev. 26, 380–392.

Suh, H.H., Bahadori, T., Vallarino, J., Spengler, J.D., 2000. Criteria air pollutants and toxic air pollutants. Environ. Health Perspect. 108, 625–633.

Takahashi, M., Fujii, J., Miyoshi, E., Hoshi, A., Taniguchi, N., 1995. Elevation of aldose reductase gene expression in rat primary hepatoma and hepatoma cell lines: implication in detoxification of cytotoxic aldehydes. Int. J. Cancer 62, 749–754.

Tesfamariam, B., Palacino, J.J., Weisbrod, R.M., Cohen, R.A., 1993. Aldose reductase inhibition restores endothelial cell function in diabetic rabbit aorta. J. Cardiovasc. Pharmacol. 21, 205–211.

Townsend, A.J., Leone-Kabler, S., Haynes, R.L., Wu, Y., Szweda, L., Bunting, K.D., 2001. Selective protection by stably transfected human ALDH3A1 (but not human ALDH1A1) against toxicity of aliphatic aldehydes in V79 cells. Chem. Biol. Interact. 130–132, 261–273.

Uchida, K., 2000. Cellular response to bioactive lipid peroxidation products. Free Radic. Res. 33, 731–737.

Uchida, K., Toyokuni, S., Nishikawa, K., Kawakishi, S., Oda, H., Hiai, H., Stadtman, E.R., 1994. Michael addition-type 4-hydroxy-2-nonenal adducts in modified low-density lipoproteins: markers for atherosclerosis. Biochemistry 33, 12487–12494.

Vander Jagt, D.L., Kolb, N.S., Vander Jagt, T.J., Chino, J., Martinez, F.J., Hunsaker, L.A., Royer, R.E., 1995. Substrate specificity of human aldose reductase: identification of 4-hydroxynonenal as an endogenous substrate. Biochim. Biophys. Acta. 1249, 117–126.

Vander Jagt, D.L., Hassebrook, R.K., Hunsaker, L.A., Brown, W.M., Royer, R.E., 2001. Metabolism of the 2-oxoaldehyde methylglyoxal by aldose reductase and by glyoxalase-I: roles for glutathione in both enzymes and implications for diabetic complications. Chem. Biol. Interact. 130–132, 549–562.

Vasiliou, V., Nebert, D.W., 2005. Analysis and update of the human aldehyde dehydrogenase (ALDH) gene family. Hum. Genomics 2, 138–143.

Vasiliou, V., Bairoch, A., Tipton, K.F., Nebert, D.W., 1999. Eukaryotic aldehyde dehydrogenase (ALDH) genes: human polymorphisms, and recommended nomenclature based on divergent evolution and chromosomal mapping. Pharmacogenetics 9, 421–434.

Vasiliou, V., Pappa, A., Estey, T., 2004. Role of human aldehyde dehydrogenases in endobiotic and xenobiotic metabolism. Drug Metab. Rev. 36, 279–299.

Vilardo, P.G., Scaloni, A., Amodeo, P., Barsotti, C., Cecconi, I., Cappiello, M., Lopez-Mendez, B., Rullo, R., Dal Monte, M., Del Corso, A., Mura, U., 2001. Thiol/disulfide interconversion in bovine lens aldose reductase induced by intermediates of glutathione turnover. Biochemistry 40, 11985–11994.

Yang, X., Zhang, Y.P., Chen, D., Chen, W.G., Wang, R., 2001. Eye irritation caused by formaldehyde as an indoor air pollution–a controlled human exposure experiment. Biomed. Environ. Sci. 14, 229–236.

Yang, Y., Sharma, R., Sharma, A., Awasthi, S., Awasthi, Y.C., 2003. Lipid peroxidation and cell cycle signaling: 4-hydroxynonenal, a key molecule in stress mediated signaling. Acta. Biochim. Pol. 50, 319–336.

Yoritaka, A., Hattori, N., Uchida, K., Tanaka, M., Stadtman, E.R., Mizuno, Y., 1996. Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. Proc. Natl. Acad. Sci. USA 93, 2696–2701.