Biochemical and Biophysical Research Communications 452 (2014) 136–141

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Reductive detoxification of acrolein as a potential role for aldehyde reductase (AKR1A) in mammals

Toshihiro Kurahashi [a], Myoungsu Kwon [a], Takujiro Homma [a], Yuka Saito [a], Jaeyong Lee [a], Motoko Takahashi [b], Ken-ichi Yamada [c], Satoshi Miyata [d], Junichi Fujii [a,*]

[a] Department of Biochemistry and Molecular Biology, Yamagata University School of Medicine, Yamagata, Japan
[b] Department of Biochemistry, Sapporo Medical University School of Medicine, Sapporo, Japan
[c] Department of Bio-functional Science, Faculty of Pharmacological Science, Kyushu University, Fukuoka, Japan
[d] Department of Internal Medicine, Osaka Hospital, Japan Community Health Care Organization, Osaka, Japan

ARTICLE INFO

Article history:
Received 27 July 2014
Available online 21 August 2014

Keywords:
Mouse embryonic fibroblast
Aldehyde reductase
Acrolein
Ascorbic acid
Detoxification

ABSTRACT

Aldehyde reductase (AKR1A), a member of the aldo–keto reductase superfamily, suppresses diabetic complications via a reduction in metabolic intermediates; it also plays a role in ascorbic acid biosynthesis in mice. Because primates cannot synthesize ascorbic acid, a principle role of AKR1A appears to be the reductive detoxification of aldehydes. In this study, we isolated and immortalized mouse embryonic fibroblasts (MEFs) from wild-type (WT) and human *Akr1a*-transgenic (Tg) mice and used them to investigate the potential roles of AKR1A under culture conditions. Tg MEFs showed higher methylglyoxal- and acrolein-reducing activities than WT MEFs and also were more resistant to cytotoxicity. Enzymatic analyses of purified rat AKR1A showed that the efficiency of the acrolein reduction was about 20% that of glyceraldehyde. Ascorbic acid levels were quite low in the MEFs, and while the administration of ascorbic acid to the cells increased the intracellular levels of ascorbic acid, it had no affect on the resistance to acrolein. Endoplasmic reticulum stress and protein carbonylation induced by acrolein treatment were less evident in Tg MEFs than in WT MEFs. These data collectively indicate that one of the principle roles of AKR1A in primates is the reductive detoxification of aldehydes, notably acrolein, and protection from its detrimental effects.

1. Introduction

Unsaturated fatty acids are prone to oxidation by reactive oxygen species (ROS), and the resultant lipid peroxidation products mostly contain aldehyde moieties. Among lipid-derived aldehydes, 4-hydroxy 2-nonenal (HNE) and malondialdehyde are abundantly produced as peroxidation products [1]. While acrolein (2-propenal) is produced to a lesser extent under oxidative conditions, it also comes mostly from tobacco smoke, heated cooking oil, and air pollutants, and it exerts the strongest cytotoxicity from among the lipid-derived aldehydes [2]. Acrolein is a major environmental risk factor for chronic obstructive pulmonary disease (COPD), and augmented levels of acrolein are found in the lung fluids of COPD patients [3]. The cytotoxic effects of acrolein are mediated by the modification of a variety of molecules including proteins and nucleic acids [4–6], which impairs glutathione homeostasis [7–9]. The formation of a DNA adduct with acrolein leads to mutagenesis and ultimately to tumorigenesis [10]. Recent studies have indicated that acrolein exerts its cytotoxicity by triggering mitochondrial damage and endoplasmic reticulum (ER) stress in pulmonary cells [11] and endothelial cells [12], in the liver [13], and in other cell lines [14]. Thus, it is intriguing to learn how acrolein can be detoxified *in vivo* from the viewpoint of health maintenance.

Enzymes in the aldo–keto reductase (AKR) superfamily catalyze the reduction of various aldehydes to their corresponding alcohols in an NADPH-dependent manner [15]. Some aldo–keto reductase family members such as aldose reductase (AKR1B), AKR1B7, AKR1C, aflatoxin B1 aldehyde reductase (AKR7A1), and AKR7A2 are reportedly involved in the detoxification of acrolein [16–21]. However, their abundance is limited in the liver, a main detoxification organ. Aldehyde reductase (AKR1A), which is highly expressed and more abundant than these family members in the liver, suppresses diabetic complications via a reduction of 3-deoxyglucosone and methylglyoxal, which are intermediates of the glycation reaction and are cytotoxic [22,23]. AKR1A is also involved in some metabolic pathways that require a reduction of the aldehyde moieties of intermediary compounds; e.g., the

* Corresponding author. Address: 2-2-2 Iidanishi, Yamagata 990-9585, Japan.

http://dx.doi.org/10.1016/j.bbrc.2014.08.072
0006-291X/

conversion of prostaglandin H_2 to prostaglandin F_2 [24]. Recently, Gabbay et al. [25] found that AKR1A and AKR1B are the enzymes responsible for the production of ascorbic acid (AsA). Although the two isozymes catalyze the same D-glucuronic acid reducing reaction process, the contribution of AKR1A to the AsA synthetic pathway is dominant due to its abundance in the liver, a primary organ in the AsA supply. Hence, AKR1A-deficient mice show severe osteopenia and spontaneous fractures due to AsA deficiency, while AKR1B-deficient mice do not. We have independently established an *Akrla* knockout mouse, along with human *Akr1a*-trangenic (Tg) mice, and demonstrated that AKR1A catalyzes the reduction of D-glucuronic acid and D-glucuronio-γ−lactone during AsA biosynthesis [26].

However, the role of AKR1A in acrolein detoxification is unclear despite the abundant expression of AKR1A in the liver [22]. Due to the broad substrate specificity, and to similarities in the enzyme characteristics, it is difficult to distinguish the individual roles of the AKR family enzymes *in vivo*. In the present study, we investigated embryonic fibroblasts (MEFs) from *Akrla*-gene modified mice as well as wild-type mice from the view-point of aldehyde toxicity and demonstrated a conceptual role of AKR1A in primates—that of acrolein detoxification.

2. Materials and methods

2.1. Chemicals

D-glucuronic acid and methylglyoxal were purchased from Sigma–Aldrich (St. Louis, MO). Acrolein was purchased from Tokyo Chemical Industry (Tokyo, Japan). D,L-glyceraldehyde was acquired from Nacalai Tesque (Kyoto, Japan). L-ascorbic acid and all other reagents were obtained from Wako Pure Chemical (Osaka, Japan).

2.2. Preparation of MEFs

E13.5 embryos from WT or Tg mice established in the previous study [26] were used for the preparation of MEFs. The head, four limbs and tail of each embryo were removed and kept for genotyping. After removal of the visceral organs, the remainder of the body was minced finely and digested with 0.1% trypsin in phosphate-buffered saline (PBS) at 37 °C for 15 min. Cells were dispersed and suspended in 10 ml of complete medium (DMEM, 100 units penicillin, 0.1 mg/ml streptomycin, 10% fetal bovine serum). The resultant cell suspension was transferred to plastic dishes and incubated in a CO_2 incubator at 37 °C.

2.3. Immortalization of MEFs by SV-40 large T antigen

Retroviruses were produced in a Phoenix helper cell line transfected with pBABE-puro SV-40 large T antigen vector (Addgene). WT and human *Akr1a*-Tg MEFs were immortalized in medium containing the retrovirus and 4 μg/ml of Polybrene (Millipore). The immortalized cells were selected by culture medium with 2.5 μg/ml of puromycin.

2.4. Cellular viability assay

MEFs grown to 80% were exposed to each aldehyde compound and cultured for 24 h. After washing with PBS, attached cells were stained with 0.1% crystal violet/1% methanol in PBS for 20 min and washed 4 times with water. The stain was solubilized in 0.5% (w/v) SDS and absorbance was recoded at $\lambda = 590$ nm in a microplate reader (Valioskan Flash, Thermo Fisher Scientific).

2.5. Protein preparation

MEFs were rinsed twice and harvested with ice-cold PBS. After centrifugation, cell pellets were lysed in RIPA buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% (w/v) Nonidet P-40, 0.5% (w/v) Deoxycholate, 0.1% SDS) containing 50 mM NaF, 2.5 mM Na-pyrophosphate, 2 mM sodium orthovanadate, 25 mM β-glycerophosphate, 40 μM APMSF, and protease inhibitor cocktail (Roche) and centrifuged at 17,400×*g* for 10 min at 4 °C. The supernatant were subjected to protein determination using a BCA kit (Pierce) followed by immunoblot analyses. For the cellular ascorbic acid and enzyme assay, cell pellets were lysed in hypotonic buffer (25 mM Hepes, pH7.5, 1 mM EDTA) with brief sonication (Microson, Misonix Inc.).

2.6. Immunoblot analyses

Aliquots of protein (40–60 μg) were separated on 7.5% or 10% SDS–polyacrylamide gels and electroblotted onto polyvinylidene difluoride (PVDF) membranes (GE Healthcare). The blots were blocked with 3% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST), and were then incubated with the polyclonal antibodies against AKR1A [22], AKR1B [27], SV40 large T antigen (sc-148, Santa Cruz), IRE1α (#3294, Cell Signaling), phosphorylated IRE1α (p-IRE1α, NB100-2323, NOVUS), CHOP (sc-7351, Santa Cruz), BiP (sc-13968, Santa Cruz), and the mouse monoclonal antibody against β-actin (sc-69879, Santa Cruz) diluted in TBST containing 1% BSA. After three washes in TBST, the blots were incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG antibody (Santa Cruz). After washing, the presence of bound horseradish peroxidase was detected by measuring chemiluminescence using Immobilon western chemiluminescent HRP substrate (Millipore) on an image analyzer (ImageQuant LAS500, GE Healthcare). A carbonyl blot was performed using an OxyBlot kit (Millipore) according to the manufacturer's instruction.

2.7. Measurement of cellular ascorbic acid

We synthesized 15-(Naphthalen-1-ylamino)-7-aza-3, 11-dioxadispiro[5.1.5^8.3^6]hexadecan-7-oxyl (Naph-DiPy) [28], for use as a fluorescent probe to measure the AsA in the cells, as described [29]. The cell lysate was incubated with Naph-DiPy for 30 min in RT. The AsA concentration was calculated by measuring the fluorescence at an excitation wavelength of 310 nm and at an emission

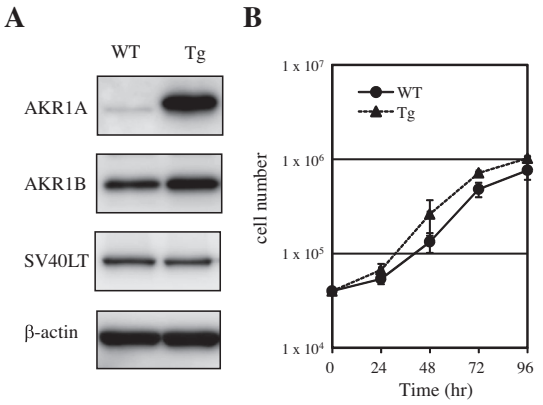

Fig. 1. Characteristics of immortalized MEFs. (A) Representative data from the immunoblots of soluble proteins from the immortalized WT and Tg MEFs. Representative data of several experiments are shown. (B) The number of cells cultivated in 3.5 cm dishes was counted at corresponding time points and is expressed per dish ($n = 3$).

wavelength of 430 nm using a microplate reader (Valioskan Flash, Thermo Fisher Scientific, Yokohama, Japan).

2.8. Assay of NADPH-dependent aldehyde-reducing activity

Aliquots of proteins from MEFs, or from recombinant rat AKR1A, were subjected to enzyme assays, as described [26]. The aldehyde-reducing activity was measured by monitoring the rate of the consumption of NADPH. The reaction mixture contained 100 mM HEPES, pH 7.4, 0.1 mM NADPH and various concentrations of aldehyde compounds. Reactions were monitored by following the decrease in absorbance of NADPH at 340 nm on a U-5100 spectrophotometer (Hitachi) at 25 °C. One unit of enzyme activity was defined as the amount of the enzyme that could catalyze the oxidation of 1 μmol of NADPH/min. Kinetic parameters were calculated from a least-squares linear regression analysis of the data.

2.9. Statistical analysis

The results are expressed as the mean ± SEM. Statistical analysis was performed using a two-tailed unpaired Student's *t* test. A *P*-value of less than 0.05 was considered significant. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. Characteristics of immortalized MEFs

In order to elucidate the role of AKR1A in cells, we isolated MEFs from WT and Tg mouse embryos and immortalized the MEFs using an SV-40 large T antigen. After consecutive cloning, we established WT and Tg MEF lines. Immunoblot analyses of the cellular extracts confirmed the overexpressed AKR1A in Tg MEF while the levels of

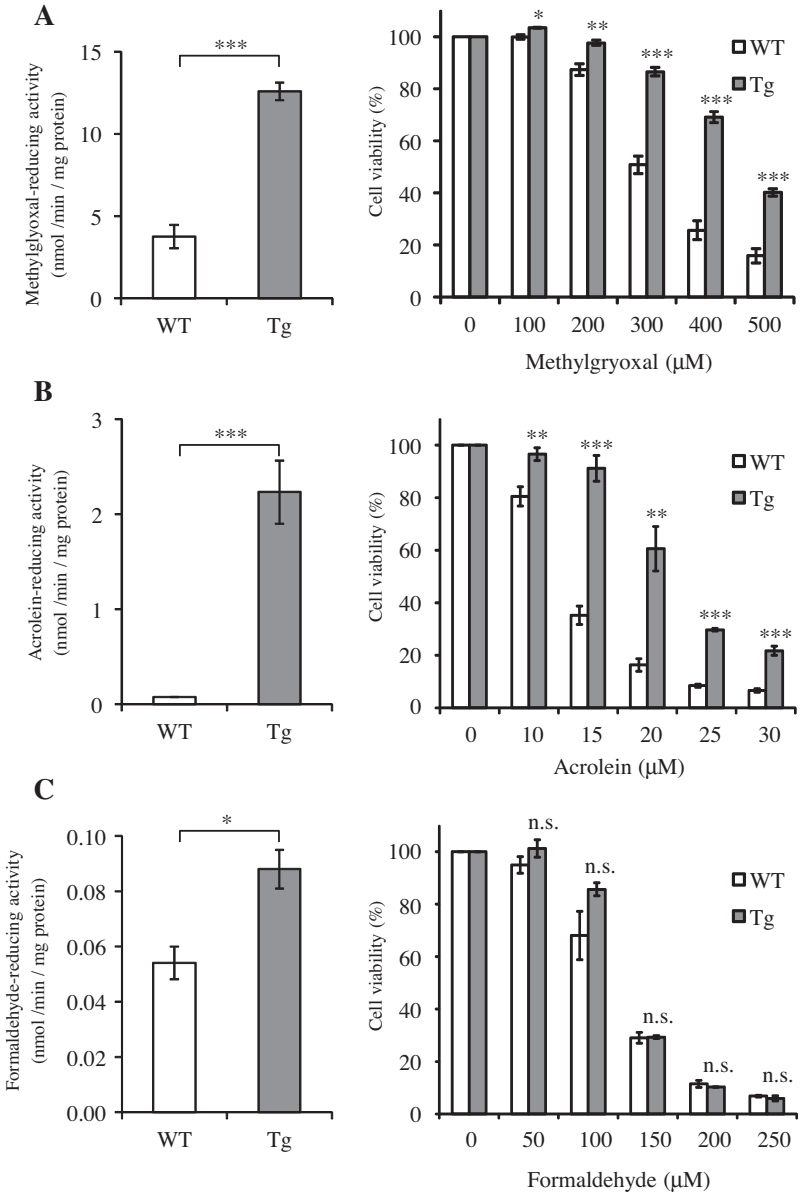

Fig. 2. Aldehyde-reducing activities and effects of aldehyde compounds on the viability of MEFs. Cytosolic proteins were extracted from the MEFs, and the NADPH-dependent reducing activities of methylglyoxal (A), acrolein (B), and formaldehyde (C) were measured by monitoring the decrease in the absorbance at 340 nm (histograms in the left). After the incubation of MEFs with various concentrations of aldehyde compounds for 24 h, cellular viability were measured (histograms appear on the right).

Table 1
Kinetics parameters of AKR1A against acrolein and formaldehyde. The values of kinetic constants were obtained at 25 °C (pH 7.4) over more than six substrate concentrations.

Substrate	K_{cat} (s^{-1})	K_m (mM)	K_{cat}/K_m (s^{-1} M^{-1})
Acrolein	0.70	2.4	290
Formaldehyde	0.71	69	10

AKR1B were the same in the WT and Tg MEFs (Fig. 1A). No significant difference was observed regarding the proliferating ability of the cells under conventional culture conditions (Fig. 1B).

3.2. Comparison of sensitivities to cytotoxic aldehyde compounds

We measured the NADPH-dependent aldehyde-reducing activities of cellular extracts from WT and Tg MEFs using methylglyoxal, acrolein, and formaldehyde, which are naturally occurring aldehydes in the body [1], as substrates. We also evaluated the cytotoxic effects of these aldehyde compounds on the MEFs. Tg MEFs showed a higher degree of methylglyoxal-reducing activity and a stronger resistance to methylglyoxal cytotoxicity compared with the WT MEFs (Fig. 2A). While acrolein was more toxic to cells than methylglyoxal, Tg MEFs again showed a higher degree of acrolein-reducing activity and a stronger resistance than WT MEFs (Fig. 2B). Formaldehyde was reduced only slightly, and no significant difference was observed between the MEFs regarding its cytotoxic effects (Fig. 2C).

We then determined the kinetic parameters for purified rat AKR1A using acrolein and formaldehyde as substrates (Table 1). Purified rat AKR1A efficiently reduced acrolein with the K_{cat}/K_m being about 29% and 3% that of the reported D,L-glyceraldehyde- and methylglyoxal-reducing activities, respectively [26], and it exhibited quite low activity when formaldehyde was used as the substrate.

3.3. Evaluation of the role of AsA in acrolein detoxification

To confirm if acrolein is actually detoxified by AKR1A in the cells, we assessed oxidative protein modification by acrolein in MEFs. Carbonyl blot analysis showed that treatment of the cells with 30 μM of acrolein for 30 min markedly elevated protein

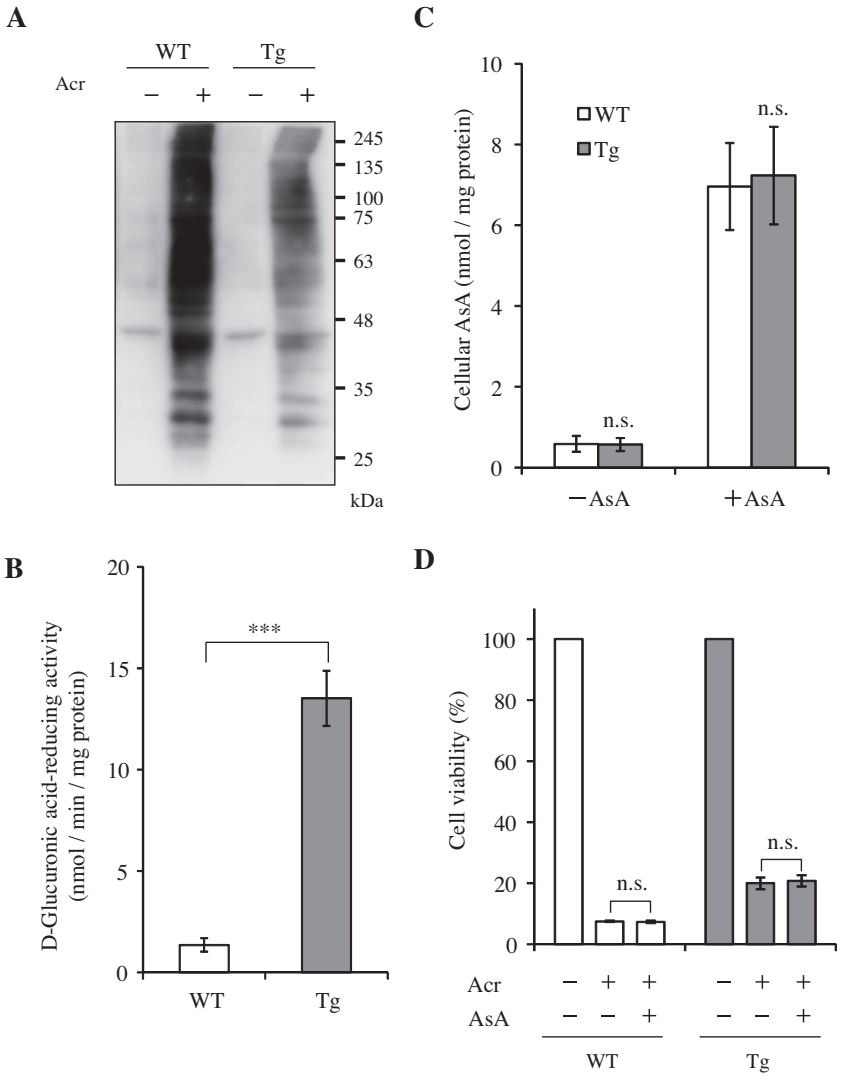

Fig. 3. AsA had no role in AKR1A-mediated cytoprotection. (A) After incubation of MEFs with or without 30 μM acrolein (Acr), protein carbonylation was assessed using an OxyBlot kit. (B) NADPH-dependent D-glucuronic acid-reducing activities for the cytosolic fractions were measured. (C) After incubation of the MEFs with or without 100 μM AsA for 4 h, cytosolic fractions were subjected to AsA assay. (D) After incubation of MEFs with or without 30 μM AsA for 24 h, the effect of 30 μM acrolein on cellular viability was examined ($n = 4$).

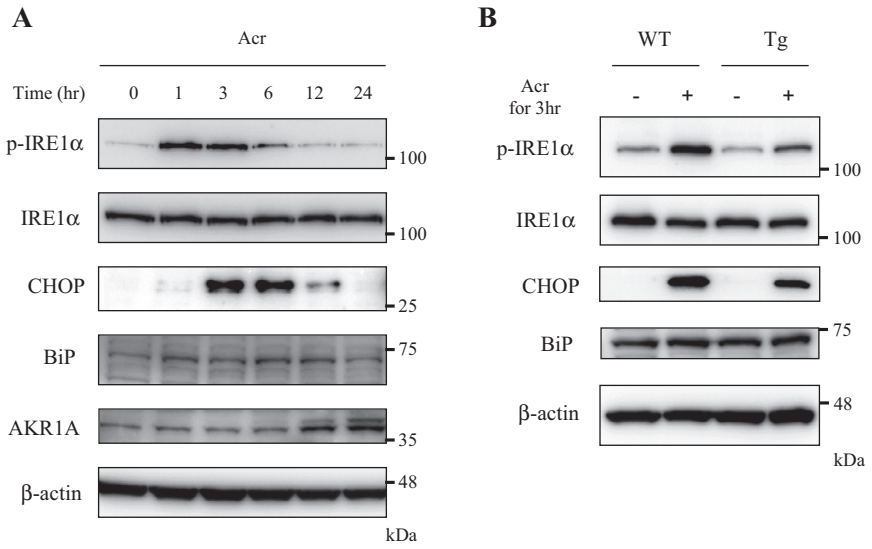

Fig. 4. Suppression of acrolein-triggered ER stress by *Akr1a* overexpression. (A) After the administration of 15 μM acrolein to WT MEF, changes in the ER stress markers were examined by immunoblot analyses. (B) After treatment of WT and Tg MEFs with 30 μM acrolein for 3 h, isolated proteins were subjected to immunoblot analyses. Representative data of several experiments are shown.

carbonyls, which were significantly suppressed in Tg MEFs (Fig. 3A). Because AKR1A is involved in AsA synthesis, we sought to determine if its cytoprotective effects were related to the production of AsA. The Tg MEFs showed a level of D-glucuronic acid-reducing activity that was much higher than that of WT MEFs (Fig. 3B), which was consistent with our previous study in liver and kidney [26]. These MEFs contained only low levels of AsA, and supplementation with 100 μM AsA to the culture media increased the intracellular AsA to a similar extent in both WT and Tg MEFs (Fig. 3C), suggesting that the AsA was properly incorporated into the cells via the AsA transporter. The low AsA content in the MEFs was not surprising because the liver is the main organ that produces AsA [30]. Thus, enzyme(s) involved in the AsA synthetic pathway were not sufficiently expressed in MEFs and hence could not support AsA synthesis. We also examined if AsA directly affected the detoxification of acrolein in MEFs and found that the inclusion of 30 μM of AsA in the culture media did not affect the cellular resistance to acrolein cytotoxicity (Fig. 3D).

3.4. AKR1A protects cells from acrolein-induced ER stress

Elevated endoplasmic reticulum (ER) stress is a hallmark of the cytotoxic effect of acrolein in a variety of cells [13]. We investigated if acrolein triggers ER stress in WT MEFs. At 15 μM, acrolein indeed increased phosphorylated IRE1α and CHOP in a few hours but had only a slight affect on BiP induction (Fig. 4A). The elevation in phosphorylated IRE1α and CHOP was suppressed in Tg MEFs compared with that in WT MEFs (Fig. 4B). It was also noteworthy that AKR1A appeared to be induced by prolonged acrolein treatment. These results collectively indicated the contribution of AKR1A enzymatic activity to the detoxification of, and resistance to, acrolein.

4. Discussion

In this communication we suggest that the detoxification of toxic aldehydes, e.g., acrolein, is a principle role for AKR1A in mammals, particularly in primates that cannot synthesize AsA. Because aldehyde compounds are reactive and hence potentially toxic to cells, the main function of AKR1A in primates appears to be one of detoxification. Despite an abundant presence in the liver, no reports have described the important role of AKR1A in acrolein detoxification.

Acrolein, a highly reactive α,β-unsaturated aldehyde, is abundantly present in tobacco smoke and also is produced in the body by lipid peroxidation reactions as well as being involved in various diseases such as COPD [21]. Some enzymes in the aldo–keto reductase superfamily, e.g., AKR1B, AKR1C, and AKR7A [16–21], reportedly detoxify acrolein. AKR1A exhibits broad substrate specificity and hence plays multiple roles by reducing the carbonyl groups of substrates. For example, AKR1A suppresses diabetic complications by reducing 3-deoxiglucosone and methylglyoxal [22,26]. A physiological role of AKR1A in AsA synthesis has been demonstrated in mice [25,26]. However, the significance of AKR1A in primates has remained obscure because the AsA synthetic pathway is defective due to a natural mutation in *Gulo* that encodes the rate-determining enzyme that is required for the final step of ascorbic acid synthesis [31].

When we investigated *Akr1a*-knockout mice over a long period, the fatal phenotype could be dramatically ameliorated by AsA supplementation, leading to the extension of a lifetime and to an improvement in female infertility [32]. However, body weight remained somewhat light, suggesting potential roles for AKR1A other than AsA synthesis. Here, we clearly demonstrated the role of AKR1A in the reductive detoxification of aldehydes, most notably acrolein. The overexpression of human *Akr1a* indeed made the Tg MEF more resistant to acrolein cytotoxicity. AKR1A reduced methylglyoxal more effectively than acrolein and formaldehyde ([26] and Table 1). However, methylglyoxal, which is present in relatively high levels *in vivo*, exhibited only moderate cytotoxicity [23]. Therefore, a concentration of methylglyoxal that was approximately one-order higher was needed in order to confirm cytotoxicity (Fig. 2A). However, acrolein is highly toxic, and it causes cell death even at a very low concentration (Fig. 2B). Thus, AKR1A showed moderate activity to acrolein compared to methylglyoxal (Table 1), but the enzyme effectively decreased the cytotoxic effects of even a low level of acrolein.

Because AsA is a potent reductant and plays multiple roles in metabolism, it was possible that it exerted protection against acrolein toxicity independent of direct reduction by AKR1A. We recently showed that the anesthetic action of pentobarbital is prolonged in *Akr1a*-deficient mice and shortened in Tg mice [29].

AKR1A activity is not directly involved in the anesthetic action of pentobarbital, however. Instead, AsA may alter the responses of the neuronal system to the anesthetic action of pentobarbital. In the present study, the inclusion of AsA in the culture media did not affect the cellular resistance to acrolein toxicity (Fig. 3D), suggesting the non-engagement of AsA in the reduced aldehyde toxicity.

Acrolein exhibits cytotoxicity by elevating reactive oxygen species and causing mitochondrial dysfunction and ER stress [13]. When acrolein is taken excessively beyond the detoxifying capacity of the cells, it causes apoptosis by increasing the reactive oxygen species [33]. We confirmed decreased levels of protein carbonylation in Tg MEFs (Fig. 3A), suggesting the suppression of oxidative stress by overexpressed *Akr1a*. An examination of the mitochondrial respiratory chain complexes by immunoblotting showed no detrimental effects of acrolein on WT or Tg MEFs (data not shown). We showed the induction of ER stress by acrolein within several hours in MEFs (Fig. 4A), and showed its amelioration by overexpressed human *Akr1a* (Fig. 4B). These data supported the notion that AKR1A indeed suppressed the cytotoxic effects of acrolein via NADPH-dependent reduction.

Here we demonstrated that AKR1A is involved in the reduction of cytotoxic aldehydes, notably acrolein, in an NADPH-dependent manner by using MEFs from genetically modified mice. Because toxic aldehyde compounds are produced by a variety of metabolic pathways and also come from environments such as tobacco smoke and air pollutants, the detoxification by AKR1A would contribute to the prevention of COPD and tumor development [3]. Thus, we unveiled the pivotal role of AKR1A in organs such as the livers of mammals.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgment

This work was supported in part by a Grant-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Sciences (Grant No. 25870075).

References

[1] R. Sultana, M. Perluigi, D. Allan Butterfield, Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain, Free Radic. Biol. Med. 62 (2013) 157–169.
[2] N. Moretto, G. Volpi, F. Pastore, F. Facchinetti, Acrolein effects in pulmonary cells: relevance to chronic obstructive pulmonary disease, Ann. N. Y. Acad. Sci. 1259 (2012) 39–46.
[3] M. Corradi, P. Pignatti, P. Manini, R. Andreoli, M. Goldoni, M. Poppa, G. Moscato, B. Balbi, A. Mutti, Comparison between exhaled and sputum oxidative stress biomarkers in chronic airway inflammation, Eur. Respir. J. 24 (2004) 1011–1017.
[4] G. Aldini, M. Orioli, M. Carini, Protein modification by acrolein: relevance to pathological conditions and inhibition by aldehyde sequestering agents, Mol. Nutr. Food Res. 55 (2011) 1301–1319.
[5] J. Wu, J.F. Stevens, C.S. Maier, Mass spectrometry-based quantification of myocardial protein adducts with acrolein in an in vivo model of oxidative stress, Mol. Nutr. Food Res. 55 (2011) 1401–1410.
[6] M. Nakamura, H. Tomitori, T. Suzuki, A. Sakamoto, Y. Terui, R. Saiki, N. Dohmae, K. Igarashi, K. Kashiwagi, Inactivation of GAPDH as one mechanism of acrolein toxicity, Biochem. Biophys. Res. Commun. 430 (2012) 1265–1710.
[7] J.P. Kehrer, S.S. Biswal, The molecular effects of acrolein, Toxicol. Sci. 57 (2000) 6–15.
[8] Y. Iuchi, T. Kaneko, S. Matsuki, T. Ishii, Y. Ikeda, K. Uchida, J. Fujii, Carbonyl stress and detoxification ability in the male genital tract and testis of rats, Histochem. Cell Biol. (2004) 123–130.
[9] H. Tomitori, M. Nakamura, A. Sakamoto, Y. Terui, M. Yoshida, K. Igarashi, K. Kashiwagi, Augmented glutathione synthesis decreases acrolein toxicity, Biochem. Biophys. Res. Commun. 418 (2012) 110–115.
[10] M.S. Tang, H.T. Wang, Y. Hu, W.S. Chen, M. Akao, Z. Feng, W. Hu, Acrolein induced DNA damage, mutagenicity and effect on DNA repair, Mol. Nutr. Food Res. 55 (2011) 1291–1300.
[11] Y. Kitaguchi, L. Taraseviciene-Stewart, M. Hanaoka, R. Natarajan, D. Kraskauskas, N.F. Voelkel, Acrolein induces endoplasmic reticulum stress and causes airspace enlargement, PLoS One 7 (2012) e38038.
[12] P. Haberzettl, E. Vladykovskaya, S. Srivastava, A. Bhatnagar, Role of endoplasmic reticulum stress in acrolein-induced endothelial activation, Toxicol. Appl. Pharmacol. 234 (2009) 14–24.
[13] M.K. Mohammad, D. Avila, J. Zhang, S. Barve, G. Arteel, C. McClain, S. Joshi-Barve, Acrolein cytotoxicity in hepatocytes involves endoplasmic reticulum stress, mitochondrial dysfunction and oxidative stress, Toxicol. Appl. Pharmacol. 265 (2012) 73–82.
[14] A. Hengstermann, T. Müller, Endoplasmic reticulum stress induced by aqueous extracts of cigarette smoke in 3T3 cells activates the unfolded-protein-response-dependent PERK pathway of cell survival, Free Radic. Biol. Med. 44 (2008) 1097–1107.
[15] M. Spite, S.P. Baba, Y. Ahmed, O.A. Barski, K. Nijhawan, J.M. Petrash, A. Bhatnagar, S. Srivastava, Substrate specificity and catalytic efficiency of aldo–keto reductases with phospholipid aldehydes, Biochem. J. 405 (2007) 95–105.
[16] R. Gardner, S. Kazi, E.M. Ellis, Detoxication of the environmental pollutant acrolein by a rat liver aldo–keto reductase, Toxicol. Lett. 148 (2004) 65–72.
[17] E.S. Kang, G.H. Kim, I.S. Woo, H.J. Kim, S.Y. Eun, S.A. Ham, H. Jin, M.Y. Kim, M.H. Park, H.J. Kim, K.C. Chang, J.H. Lee, J.H. Kim, C. Yabe-Nishimura, H.G. Seo, Down-regulation of aldose reductase renders J774A.1 cells more susceptible to acrolein- or hydrogen peroxide-induced cell death, Free Radic. Res. 42 (2008) 930–938.
[18] D. Li, M. Ferrari, E.M. Ellis, Human aldo–keto reductase AKR7A2 protects against the cytotoxicity and mutagenicity of reactive aldehydes and lowers intracellular reactive oxygen species in hamster V79-4 cells, Chem. Biol. Interact. 195 (2012) 25–34.
[19] R.C. Lyon, D. Li, G. McGarvie, E.M. Ellis, Aldo–keto reductases mediate constitutive and inducible protection against aldehyde toxicity in human neuroblastoma SH-SY5Y cells, Neurochem. Int. 62 (2013) 113–121.
[20] W.N. Jagoe, K. Howe, S.C. O'Brien, J. Carroll, Identification of a role for a mouse sperm surface aldo–keto reductase (AKR1B7) and its human analogue in the detoxification of the reactive aldehyde, acrolein, Andrologia 45 (2013) 326–331.
[21] U.C. Yadav, K.V. Ramana, S.K. Srivastava, Aldose reductase regulates acrolein-induced cytotoxicity in human small airway epithelial cells, Free Radic. Biol. Med. 65 (2013) 15–25.
[22] M. Takahashi, J. Fujii, T. Teshima, K. Suzuki, T. Shiba, N. Taniguchi, Identity of a major 3-deoxyglucosone-reducing enzyme with aldehyde reductase in rat liver established by amino acid sequencing and cDNA expression, Gene 127 (1993) 249–253.
[23] A. Okado, Y. Kawasaki, Y. Hasuike, M. Takahashi, T. Teshima, J. Fujii, N. Taniguchi, Induction of apoptotic cell death by methylglyoxal and 3-deoxyglucosone in macrophage-derived cell lines, Biochem. Biophys. Res. Commun. 225 (1996) 219–224.
[24] H. Hayashi, Y. Fujii, K. Watanabe, Y. Urade, O. Hayaishi, Enzymatic conversion of prostaglandin H2 to prostaglandin F2 alpha by aldehyde reductase from human liver: comparison to the prostaglandin F synthetase from bovine lung, J. Biol. Chem. 264 (1989) 1036–1040.
[25] K.H. Gabbay, K.M. Bohren, R. Morello, T. Bertin, J. Liu, P. Vogel, Ascorbate synthesis pathway: dual role of ascorbate in bone homeostasis, J. Biol. Chem. 285 (2010) 19510–19520.
[26] M. Takahashi, S. Miyata, J. Fujii, Y. Inai, S. Ueyama, M. Araki, T. Soga, R. Fujinawa, C. Nishitani, S. Ariki, T. Shimizu, T. Abe, Y. Ihara, M. Nishikimi, Y. Kozutsumi, N. Taniguchi, Y. Kuroki, In vivo role of aldehyde reductase, Biochim. Biophys. Acta 2012 (1820) 1787–1796.
[27] M. Takahashi, J. Fujii, E. Miyoshi, A. Hoshi, N. Taniguchi, Elevation of aldose reductase gene expression in rat primary hepatoma and hepatoma cell lines: implication in detoxification of cytotoxic aldehydes, Int. J. Cancer 62 (1995) 749–754.
[28] Y. Matsuoka, M. Yamato, T. Yamasaki, F. Mito, K. Yamada, Rapid and convenient detection of ascorbic acid using a fluorescent nitroxide switch, Free Radic. Biol. Med. 53 (2012) 2112–2118.
[29] J. Ito, N. Otsuki, X. Zhang, K. Konno, T. Kurahashi, M. Takahashi, M. Yamato, Y. Matsuoka, K. Yamada, S. Miyata, J. Fujii, Ascorbic acid reverses the prolonged anesthetic action of pentobarbital in Akr1a-knockout mice, Life Sci. 95 (2014) 1–8.
[30] C.L. Linster, E. Van Schaftingen, Vitamin C. Biosynthesis, recycling and degradation in mammals, FEBS J. 274 (2007) 1–22.
[31] M. Nishikimi, R. Fukuyama, S. Minoshima, N. Shimizu, K. Yagi, Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-gamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man, J. Biol. Chem. 269 (1994) 13685–13688.
[32] H. Nishida, T. Kurahashi, Y. Saito, N. Otsuki, M. Kwon, H. Ohtake, M. Yamakawa, K. Yamada, S. Miyata, Y. Tomita, J. Fujii, Kidney fibrosis is independent of the amount of ascorbic acid in mice with unilateral ureteral obstruction, Free Radic. Res. 48 (2014) 1115–1124.
[33] J. Roy, P. Pallepati, A. Bettaieb, A. Tanel, D.A. Averill-Bates, Acrolein induces a cellular stress response and triggers mitochondrial apoptosis in A549 cells, Chem. Biol. Interact. 181 (2009) 154–167.