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Note

Evaluation of quercetin as a potential cytoprotector against acetaldehyde using the cultured hepatocyte model with aldehyde dehydrogenase isozyme deficiency

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Abstract

Protective effect of quercetin against acetaldehyde was evaluated using the cultured hepatocyte models with aldehyde dehydrogenase (ALDH) isozyme deficiency (*aldh2-kd* and *aldh1a1-kd*). The quercetin-induced cytoprotection against acetaldehyde in the ALDH1A1-deficient mutant (*aldh1a1-kd*) was weaker than that in the wild type. Furthermore, quercetin did not enhance the ALDH activity in *aldh1a1-kd* cells, suggesting that ALDH1A1 is involved in quercetin-induced cytoprotection.

Keywords: quercetin, acetaldehyde, aldehyde dehydrogenase, cytoprotection

Acetaldehyde is the major ethanol metabolite closely related to several abnormal behaviors and chronic diseases induced by ethanol (Rocco *et al.* 2014). Acetaldehyde directly attacks the amino acid residues of proteins and DNA by its own electrophilic reactivity, and forms covalent conjugates (Ceni, Mello and Galli 2014). The excessive accumulation of acetaldehyde is also linked to mitochondria dysfunction and excessive ROS production (Zhang *et al.* 2023). These events are suggested to be mainly involved in alcohol-related toxic phenomena.

Aldehyde dehydrogenases (ALDHs), a major enzyme superfamily responsible for the oxidation of endogenous and exogenous aldehydes, play an important role in ethanol metabolism and acetaldehyde detoxification (Vasiliou and Nebert 2005). Among the 19 human ALDHs, ALDH2, a mitochondrial enzyme highly expressed in the liver, mainly contributes to acetaldehyde metabolism. The mutation (E504K) of the *ALDH2* gene (*ALDH2**2) dramatically reduces the enzyme activity compared to the wild type (WT) ALDH2. Persons with even one *ALDH2**2 allele have a 16% ALDH activity of normal individuals with 10-fold higher plasma acetaldehyde levels (Edenberg and McClintick 2018). This genetic trait, which is closely related to alcohol intolerance and dependence, is region-specific, since the *ALDH2**2-positive rate in the East Asian populations, including the Japanese, is about 40%, whereas it is less than 5% in Europeans and Africans. Notably, drinking habits of people with the *ALDH2**2 allele increase the developmental risk of upper aerodigestive, gastric, and hepatic cancers (Edenberg and McClintick 2018). In addition to ALDH2, ALDH1A1, a cytosolic enzyme expressed in the liver, plays a supportive role in acetaldehyde metabolism. ALDH1A1 is also implicated in alcohol-induced flushing and alcohol sensitivity in Caucasians (Lind, Eriksson and Wilhelmsen 2008). Therefore, it is highly important to prevent alcohol-intolerant individuals with ALDH isozyme deficiency from the symptoms of alcohol intolerance and alcohol-induced diseases by using natural phytochemicals that increase the ALDH enzyme activity.

Quercetin, a major dietary flavonoid ubiquitously found in fruits and vegetables, has been expected to contribute to human health promotion and disease prevention due to its diverse biological activities, including radical scavenging and antioxidative activities (Russo *et al.* 2012). Quercetin was also reported to mitigate alcohol-induced liver injury in a mouse model (Zhu, Zhou and Zhao 2017). Although quercetin is a promising phytochemical that prevents alcohol-related diseases in humans, it is still unclear whether quercetin protects the hepatocytes from acetaldehyde-induced cytotoxicity. In the present study, the protective effect of quercetin was evaluated using the cultured hepatocyte models with the ALDH isozyme deficiency unique to the East Asian and Caucasian populations. The ALDH isozyme-deficient mutants (*aldh2-kd* and *aldh1a1-kd*) were established via CRISPR/Cas9 genome editing. The

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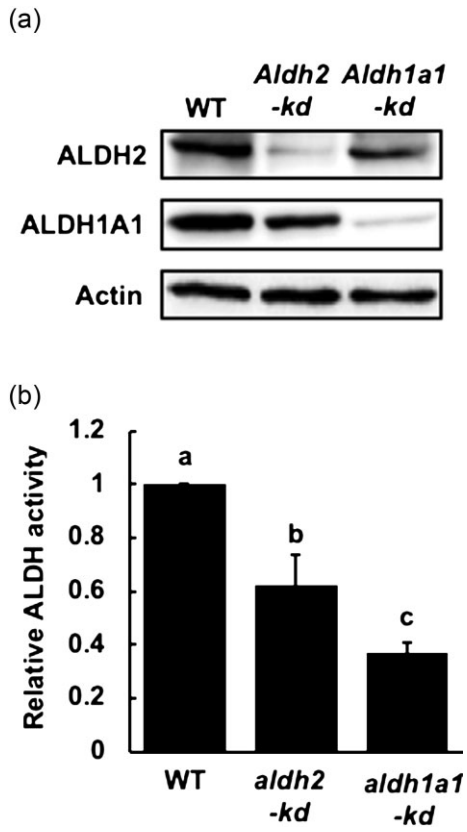

Figure 1. Knockdown effect of CRISPR/Cas9 on the ALDHs expression and ALDH activity. (a) The ALDH2 and ALDH1A1 expression levels in the WT, *aldh2-kd*, and *aldh1a1-kd* HepG2 cells were checked by a Western blot analysis. β-Actin was used as the endogenous control. (b) The total ALDH activity was determined by the NADH spectrophotometric method. All values are expressed as means ± SD of three separate experiments and analyzed by a one-way ANOVA followed by Tukey's HSD using SPSS software. The different letters above the bars indicate significant differences among the treatments for each condition ($P < .05$).

pretreatment of quercetin significantly inhibited the acetaldehyde-induced cytotoxicity not only in the WT cells, but also in each mutant. We also demonstrated here that quercetin enhanced the total ALDH activity through the ALDH1A1-dependent mechanism.

Two pools of *aldh2-kd* and *aldh1a1-kd* HepG2 cells were established by infection with lentivirus plasmid containing the ALDH2-KO CRISPR/Cas9 plasmid or ALDH1A1-KO CRISPR/Cas9 plasmid into HepG2 cells followed by puromycin selection and limiting dilution. At the protein level, we confirmed that the ALDH2 and ALDH1A1 protein expression was drastically lost in the *aldh2-kd* and *aldh1a1-kd* cells, respectively (Figures 1a and S1). On the other hand, the total ALDH activity was reduced to a greater extent in the *aldh1a1-kd* cells (37% of WT) than in the *aldh2-kd* cells (62% of WT) (Figure 1b). The two mutant cells exhibited no obvious morphological change and showed a proliferation speed similar to the WT cells as previously reported (Wang *et al.* 2018). We also confirmed that there were no significant differences in growth rate between the 3 cell lines (Figure S2). Amplicon sequencing revealed that the knockdown populations were not homogeneous, whereas the *aldh2-kd* cells having the sequence "TTGCGCGCTGCCGCC-(28 base-deletion)-TCTT" and the *aldh1a1-kd* cells having the sequences "CTCG-(1 base-deletion)-AAGCATCCATAGTAC" and "CTCGG-(G, 1 base-insertion)-AAGCATCCATAGTAC" at the target sites were predominant (Figure S3). Although these genomic frame shifts could induce the protein translation deficiency, further experiments using high homogeneous knockdown clones will be needed to support our findings.

We examined whether the quercetin pretreatment inhibited the acetaldehyde-induced cytotoxicity in the WT and *aldh2-kd* cells. We initially observed that the 3-h incubation of 10 mM acetaldehyde decreased the cell viability in both the WT and *aldh2-kd* cells (Figure 2a). There is a significant difference in acetaldehyde sensitivity at 10 mM between the WT and *aldh2-kd* cells (Figure 2a). The 6-h pretreatment of quercetin significantly inhibited the acetaldehyde-induced cytotoxicity at 5 µM in the WT cells and at 10 µM in *aldh2-kd* cells (Figure 2a). On the other hand, the two cell lines showed significant differences in cell viability at the same quercetin concentration (Figure 2a). Furthermore, the 6-h treatment of 5 µM quercetin alone significantly enhanced the total ALDH activity with propionaldehyde as a substrate not only in the WT cells, but also in the *aldh2-kd* cells (Figure 2c). These results suggested that ALDH2 might contribute to basal acetaldehyde sensitivity rather than the quercetin-induced cytoprotection.

As for the *aldh1a1-kd* cells, the treatment of 10 mM acetaldehyde for 3 h significantly decreased the cell viability as in the WT cells (Figure 2b). The quercetin pretreatment significantly inhibited the acetaldehyde-induced cytotoxicity at the concentration of 10 µM in the *aldh1a1-kd* cells, whereas, in this experiment, the significant protection in the WT cells was observed at 2.5 µM of quercetin (Figure 2b). Furthermore, the quercetin treatment significantly enhanced the total ALDH activity in the WT cells, whereas it did not in the *aldh1a1-kd* cells (Figure 2c). These results suggested that ALDH1A1, at least in part, plays an important role in quercetin-induced cytoprotection as well as enhancement of the total ALDH activity. Since quercetin enhanced the resistance against acetaldehyde in the *aldh1a1-kd* cells without the elevation of the ALDH activity, the quercetin-induced cytoprotection might involve the intracellular antioxidant enzyme-dependent pathways other than the ALDH-dependent mechanism (Li *et al.* 2022; Wu *et al.* 2023).

In the present study, we established the ALDH isozyme-deficient mutants (*aldh2-kd* and *aldh1a1-kd*) via CRISPR/Cas9 genome editing. These knockdown cells have the potential to serve as cultured cell models for region-specific alcohol intolerance. Unexpectedly, the total ALDH activity was more profoundly reduced in the *aldh1a1-kd* cells than in the *aldh2-kd* cells (Figure 1b), suggesting that, under the experimental conditions of this study, ALDH1A1 is mainly responsible for the total ALDH activity with propionaldehyde as a substrate. The present results also suggested that quercetin has the potential to protect against the acetaldehyde-induced cytotoxicity in human hepatocytes through the enhancement of the total ALDH activity. The minimum concentration of quercetin required for this effect (5 µM, Figure 2c) was lower than those of phenolic acids (Liu *et al.* 2017; Liu *et al.* 2022), suggesting that quercetin is another potential enhancer of the ALDH activity. Since there was no enhancement of the total ALDH activity in *aldh1a1-kd* cells (Figure 2c) and the cytoprotective effect in *aldh1a1-kd* cells was weaker than in the WT cells (Figure 2b), quercetin is a potential candidate for improving the symptoms caused by ALDH2 polymorphism-dependent alcohol intolerance, which is unique to East Asians.

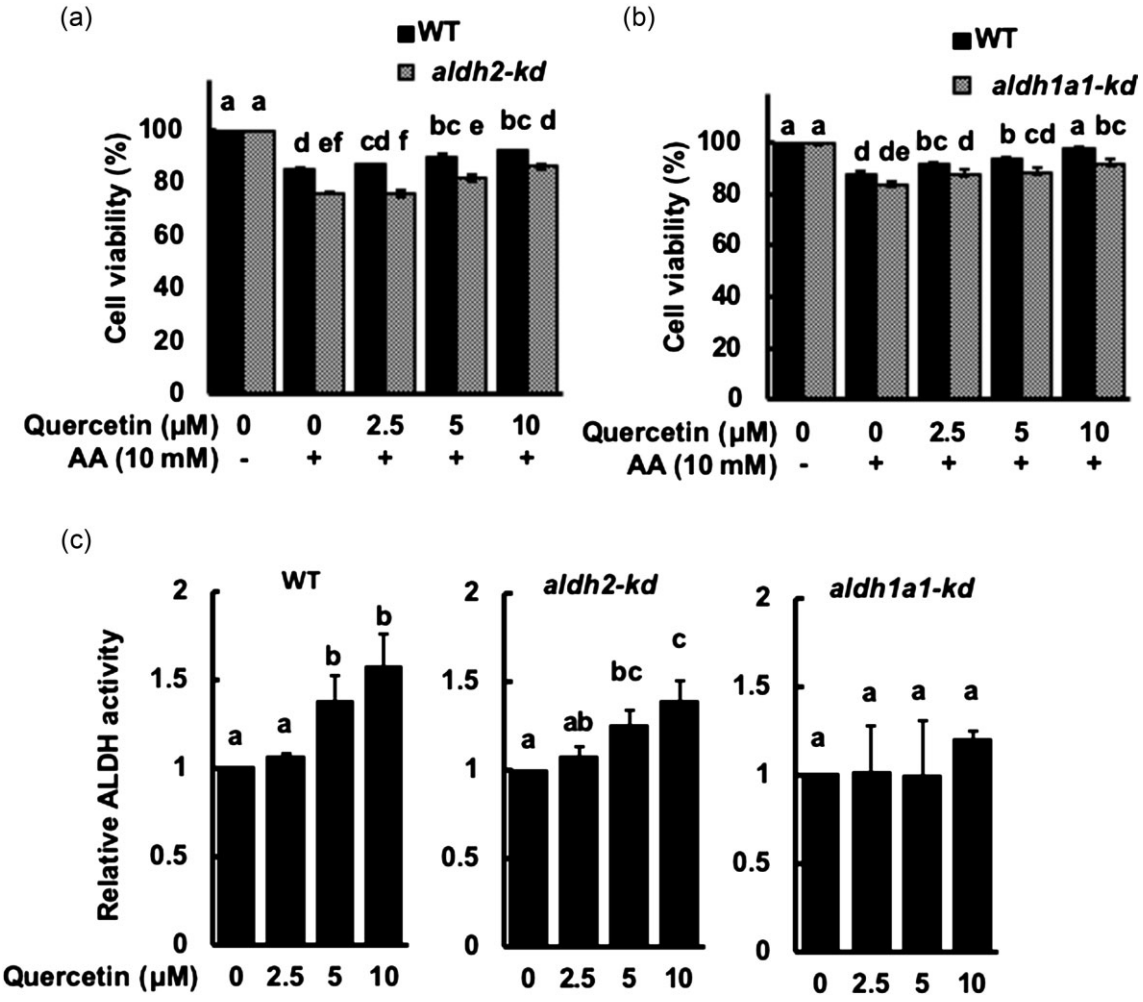

Figure 2. Difference in the quercetin-induced cytoprotection and ALDH activity enhancement between the WT, *aldh2-kd* and *aldh1a1-kd* cells. (a) Inhibitory effect of quercetin on the acetaldehyde-induced cytotoxicity in the WT and *aldh2-kd* cells. After the WT and *aldh2-kd* cells were pretreated with quercetin for 6 h, the cells were treated with 10 mM acetaldehyde for 3 h, then an MTT assay was carried out. (b) Inhibitory effect of quercetin on the acetaldehyde-induced cytotoxicity in the WT and *aldh1a1-kd* cells. (c) Enhancing effect of quercetin on the total ALDH activity. After the treatment of quercetin for 6 h, the cells were harvested, then the total ALDH activity was determined by the NADH spectrophotometric method. All values are expressed as means ± SD of 3 separate experiments and analyzed by a one-way ANOVA followed by Tukey's HSD using SPSS software. The different letters above the bars indicate significant differences among the treatments for each condition ($P < .05$).

Supplementary material

Supplementary material is available at *Bioscience, Biotechnology, and Biochemistry* online.

Data availability

The data will be shared on reasonable request to the corresponding author.

Author contribution

A.S., A.M., T.N., and Y.N. designed the project. Y.X., T.S., R.S., A.I., and A.S. conducted the experiments and analyzed the data. A.M., S.M., Y.M., T.N. and Y.N. assisted with the experiments, interpreted the data and contributed to the discussions. Y.X. and Y.N. drafted the manuscript. T.N. and Y.N. supervised and edited the paper. All authors critically read and contributed to this manuscript.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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