Effects of acetaldehyde inhalation in mitochondrial aldehyde dehydrogenase deficient mice (Aldh2\(^{-/-}\))

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1. ABSTRACT

Human body might be exposed to acetaldehyde from smoking or occupational environment, which is known to be associated with cancer through the formation of DNA adducts, in particular, \(\text{N}^2\)-ethylidene-\(\text{dG}\). Aldehyde dehydrogenase 2 (ALDH2) is the major enzyme that contribute to the detoxification of acetaldehyde in human body. In this study, wild type (Aldh2\(^{+/+}\)) and Aldh2KO (Aldh2\(^{-/-}\)) mice were exposed to the air containing 0, 125, 500 ppm acetaldehyde for 2 weeks. After inhalation, levels of \(\text{N}^2\)-ethylidene-\(\text{dG}\) in the chromosomal DNA were analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS). \(\text{N}^2\)-ethylidene-\(\text{dG}\) levels in livers of Aldh2\(^{-/-}\) mice were always lower than those of Aldh2\(^{+/+}\) mice, suggesting that Aldh2 deficiency might cause the induction of acetaldehyde metabolizing enzymes in the liver such as P450s. The differences between Aldh2\(^{-/-}\) and Aldh2\(^{+/+}\) mice were greater in the order of nasal epithelium > lung > dorsal skin, suggesting that nasal epithelium and lung are the major target sites for acetaldehyde. Acetaldehyde inhalation may cause a high risk in nasal epithelium and lung cancers for individuals with inactive ALDH2.

2. INTRODUCTION

Alcohol misuse is linked to a variety of social and medical problems. The number of Japanese alcoholism patients was about 2.5 million in 1995 and has been gradually increasing (1, 2). Alcohol misuse affects many organs, and is associated with the incidence of various cancers, such as esophageal cancer (3-5). Epidemiological evidence indicates that alcohol consumption is related to the development of various cancers and liver diseases, all of which are associated with altered levels of various intracellular oxidizing enzymes (6-8). Therefore, the metabolic pathway of ethanol and its variants among individuals are of great interest for risk assessment and prevention of diseases caused by alcohol abuse.

Ingested ethanol is oxidized by cytosolic class I alcohol dehydrogenase 2 (ADH2) to acetaldehyde, which is subsequently oxidized by mitochondrial aldehyde dehydrogenase 2 (ALDH2) to produce non-toxic acetate (9, 10). Human ALDH isozymes are divided into two groups determined by their Michaelis constant values for acetaldehyde; the low \(K_m\) ALDH (ALDH1 and ALDH2), and high \(K_m\) ALDH (ALDH3 and ALDH4). The \(K_m\) values of ALDH3 and ALDH4 are in millimolar (5–83 mM) (11),
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**Figure 1.** Formation of acetaldehyde-dG adducts. The reaction of deoxyguanosine with acetaldehyde produces $N^2$-ethylidene-2'-deoxyguanosine. The product further reacts with acetaldehyde and produces $\alpha$-methyl-$\gamma$-hydroxy-1,$N^2$-propano-2'-deoxyguanosine. To determine the amount of $N^2$-ethylidene-2'-deoxyguanosine ($N^2$-ethylidene-dG), its reduced product, $N^2$-ethyl-2'-deoxyguanosine ($N^2$-Et-dG), is analyzed by LC/MS/MS as described in “Materials and Methods”.

Cytosolic ALDH1 in micromolar (180 µM), and mitochondrial ALDH2 in nanomolar (200 nM) (12), suggesting that ALDH2 is a key enzyme responsible for catalyzing oxidation acetaldehyde in human liver.

**ALDH2*2** is a genetic polymorphism of ALDH2 resulting in an amino acid substitution from glutamic acid at 487 to lysine (E487K), and is particularly prevalent in Asian populations (13). ALDH2 functions as a homotetramer, and the inactive subunit produced by the **ALDH2** allele acts in a dominant negative fashion. It is predicted that individuals who possess the **ALDH2*1/2*2** genotype will have only 6.25% of the normal ALDH2 protein and that other tetramers containing one or more of the **ALDH2*2** subunits are mostly inactive. However, when taken together, the overall measured activity of the five possible tetramer combinations of the **ALDH2*1/2*2** genotype is approximately 13% (14). On the other hand, individuals who are **ALDH2*2/2*2** homozygous have no ALDH2 activity.

Individuals with **ALDH2*2** allele show high blood acetaldehyde concentrations after intake of only a moderate amount of alcohol (2). Acetaldehyde itself is a carcino gen that induces nasal tumors in experimental animals by inhalation (15), and is thought to be a tumor-initiator because of its mutagenic and DNA-damaging properties (16-19). It has been confirmed that acetaldehyde associated with alcoholic beverages is carcinogenic to human (Group I) (20). As a consequence of the decreased acetaldehyde metabolism, the **ALDH2*2** allele is associated with alcohol-induced flushing, and is also positively related to hepatocellular carcinoma (21, 22), oral cancer and esophageal cancer (5), while it negatively affects coronary heart disease (2, 23).

Recently, an analytical method was developed for the quantitative determination of acetaldehyde-derived stable DNA adducts, $N^2$-ethyl-2'-deoxyguanosine ($N^2$-Et-dG), $\alpha$-S- and $\alpha$-R-methyl-$\gamma$-hydroxy-1,$N^2$-propano-2'-deoxyguanosine ($\alpha$-S-Me-$\gamma$-OH-PdG and $\alpha$-R-Me-$\gamma$-OH-PdG) using sensitive liquid chromatography tandem mass spectrometry (LC/MS/MS) (24, 25). Other than these stable DNA adducts, the reaction of acetaldehyde with dG results in the formation of an unstable Schiff base at the $N^2$ position of dG ($N^2$-ethylidene-dG) (Figure 1). Wang et al. showed that $N^2$-ethylidene-dG in human liver DNA is relatively stable and the presence of this adduct could be confirmed by detection of $N^2$-Et-dG after reduction of DNA during isolation and enzymatic hydrolysis (26). They showed that when the reduction step was included during these steps, a few 100 times more $N^2$-Et-dG was detected in some cases.
3. MATERIALS AND METHODS

3.1. Wild type (Aldh2+/+ mice) and Aldh2KO mice (Aldh2−/− mice)

Male C57BL/6 (Aldh2+/+) mice, at 10 weeks of age, were purchased from Charles River Japan, Inc. (Yokohama) and male Aldh2 KO (Aldh2−/−) mice, at 10 weeks of age, were generated as previously described (27). Aldh2−/− mice were back-crossed with C57BL/6 strain for more than 10 generations. These mice were housed in specific pathogen-free units of the Division of Animal Care at the University of Occupational and Environmental Health. Seven or ten mice were placed in polycarbonate cage (W215xH140xD320 mm). Mice were adjusted to the new environment for a week before use. Autoclaved cages, floor beds, and rodent chow were used. The mice cage was cleaned every day. All the mice were treated in accordance with the guidelines of the Animal Welfare and approved by the Ethics Committee of the Animal Care and Experimentation of the UOEH (28-30).

3.2. Treatments

A group of 10 mice of each Aldh2+/+ and Aldh2−/− were exposed to filtered atmospheric air (0 ppm) and were exposed to air containing 500 ppm acetaldehyde (500 ppm exposed-group). A second group of seven of each Aldh2−/− mice were exposed to filtered atmospheric air (0 ppm) and a third group of ten of each Aldh2−/− group of ten of each 125 ppm acetaldehyde (125 ppm exposed-group). DNA adducts in target organs of Aldh2KO mice served as controls. A second group of seven of each Aldh2−/− mice were exposed to filtered atmospheric air (0 ppm) and were exposed to air containing 500 ppm acetaldehyde (500 ppm exposed-group). Mice were divided randomly and the mice were sacrificed at the end of the experiment. During this period body weights were recorded every day and the mice were sacrificed at the end of the experiment.

3.3. Blood acetaldehyde concentration

Mouse blood was collected from the decapitated trunk into liquid nitrogen-cooled plastic tubes and stored. The blood (0.5mL) was transferred into ice cold 0.6N perchloric acid solution (PCA) and centrifuged. Acetaldehyde concentration was measured as previously described (28, 31), using Hewlett-Packard headspace sampler (HP7694; Wilmington, DE) and Hewlett-Packard gas chromatograph (HP6890, Wilmington, DE) equipped with a 60 m x 0.25 mm inner diameter AQUATIC capillary column (GL Sciences, Tokyo, Japan) with a film thickness of 1.0 µm that was connected to a mass spectrometer (JOEL JMS-BU20, Tokyo, Japan).

3.4. DNA isolation from mouse organs

For quantification of N2-Et-dG, DNA was isolated from mouse organs using Gentra Puregen tissue kit as described previously (24). The procedure was basically carried out as per manufacturer’s instructions except the addition of NaBH4-CN to all solutions (final concentration: 100 mM). After purification, DNA was dissolved in 10 mM Tris-HCl/5 mM EDTA buffer (pH 7.0), extracted with chloroform and precipitated with ethanol.

3.5. DNA digestion (25)

20 µg aliquots of DNA were digested into their constituent 2’-deoxyribonucleoside-3’-monophosphate units by adding 15µl of 17 mM citrate plus 8 mM CaCl2 buffer that contained micrococcal nuclease (22.5 U) and spleen phosphodiesterase (0.075 U) plus internal standards. The solutions were mixed and incubated for 3 h at 37 °C, after which alkaline phosphatase (1 U), 10µl of 0.5 M Tris-HCl (pH 8.5), 5µl of 20 mM ZnSO4 and 67µl of distilled water were added and incubated for further 3 h at 37 °C. The digested sample was extracted twice with methanol. The methanol fractions were evaporated to dryness, resuspended in 50 µl of distilled water and subjected to liquid chromatography tandem mass spectrometry (LC/MS/MS).

3.6. Instrumentation (25)

LC/MS/MS analyses were performed using a Shimadzu LC system (Shimadzu, Kyoto, Japan) interfaced with a Quattro Ultima triple stage quadrupole MS (Waters-Micromass, Manchester, UK). The LC column was eluted over a gradient that began at a ratio of 5% methanol to 95% water and was changed to 40% methanol over a period of 30 min, changed to 80% methanol from 30 to 35 min, and finally returned to the original starting conditions, methanol: H2O = 5:95, for the remaining 11 min. The total run time was 46 min. Sample injection volumes of 20 µl each were separated on a Shim-pack XR-QDS column (3.0 mm×75 mm, 2.2 µm) and eluted at a flow rate of 0.2 ml/min. Mass spectral analyses were carried out in positive ion mode with nitrogen as the nebulizing gas. The ion source temperature was 130 °C, the desolvation gas temperature was 380 °C and the cone voltage was operated at a constant 35 V. Nitrogen gas was also used as the desolvation gas (700 l/h) and cone gas (35 l/h), and argon was used to provide a collision cell pressure of 1.5 × 103 mbar. Positive ions were acquired in multiple reactions monitoring (MRM) mode. The MRM transitions monitored were as follows: [15N5]2-oxo-dG, m/z 288 → 172; 2-oxo-dG, m/z 283 → 167; [15N5] N2-Et-dG, m/z 301→ 185 and N2-Et-dG, m/z 295.5 → 179.9. The amount of each adduct was quantified by the ratio of the peak area of the target adducts to that of its stable isotope. Quanlynx (version 4.0) software (Waters-Micromass) was used to create standard curves and to calculate adduct concentrations. The amount of deoxyguanosine was monitored at 254 nm by a Shimadzu SPD-10A UV-Visible detector that was in place before the tandem MS.

3.7. Statistics

Analysis of co-variance was carried out on body weights. For changes of DNA adduct levels, the chi-square test was used.
During the purification step, and converted to stable Et-dG derived from lysis buffer containing the strong reducing agent NaBH3CN, chromosomal DNA, tissue samples were homogenized in 4.2. DNA adduct levels of target organs in mice treated with acetaldehyde inhalation.

In the nasal epithelium of 125 ppm groups, the N2-ethylidene-dG levels were much higher than in other organs of both Aldh2+/+ and Aldh2-/- mice. In the nasal epithelium, the N2-ethylidene-dG level of Aldh2+/+ mice (n=3, 581 ± 61.4) was greatly increased compared with Aldh2-/- mice (n=3, 193 ± 158) (Figure 4).

In the lung of 125 ppm groups, Aldh2+/+ mice showed a higher level of N'-ethylidene-dG (n=7, 68.4 ± 12.4) than Aldh2-/- mice (n=7, 54.7 ± 8.39) as shown in Figure 5; this difference is statistically significant. In 500 ppm groups, the difference between Aldh2+/+ (n=10, 283 ± 82.3) and Aldh2-/- mice (n=10, 171 ± 49.8) was greater than that observed in the 125 ppm groups. In the lung, dramatically increased levels of N'-ethylidene-dG were observed in the 500 ppm groups only, in both Aldh2+/+ and Aldh2-/- mice although it occurred in the 125 ppm groups as well in the nasal epithelium.

Comparing with control group after treatment and 500 ppm exposure group before treatment (p<0.01)

4. RESULTS


The average actual concentration of 125 ppm exposure groups was 126.3 ppm, and the actual exposure of 500 ppm groups was 510.5 ppm. The mean blood acetaldehyde concentration of Aldh2+/+ mice (n=3) and Aldh2-/- mice (n=3) in the 125 ppm exposed-groups were 1.65 μM and 2.39 μM, respectively. Those of Aldh2+/+ mice (n=3) and Aldh2-/- mice (n=3) in the 500 ppm exposed-groups were 1.72 μM and 8.90 μM, respectively. The mean blood acetaldehyde concentration of Aldh2-/- mice was more than five times as high as that of Aldh2+/+ mice in the 500 ppm exposed-groups. As shown in Table 1, the mean body weight of 500 ppm exposed-groups was significantly reduced after treatment, although control and 125 ppm exposed-groups did not show any visible weight loss (p < 0.01).

4.2. DNA adduct levels of target organs in mice treated with acetaldehyde inhalation.

To determine the level of N'-ethylidene-dG in chromosomal DNA, tissue samples were homogenized in lysis buffer containing the strong reducing agent NaBH3CN, followed by DNA purification in the presence of NaBH3CN. During the purification step, N'-ethylidene-dG is reduced and converted to stable N2-Et-dG that can be sensitively quantified (24). Typical LC/MS/MS-chromatograms of N2-Et-dG derived from N2-ethylidene-dG in nasal epithelium DNA of 125 ppm exposure groups were shown in Figure 2. The N2-ethylidene-dG levels of nasal epithelium DNA from Aldh2+/+ mice was more than eight times higher than that observed in Aldh2-/- mice exposed to 125 ppm. In the liver, the average N2-ethylidene-dG levels of DNA from Aldh2+/+ mice (n=10) and Aldh2-/- mice (n=10) of 0 ppm groups were 62.4 ± 31.0 and 40.6 ± 26.7 adducts per 108 bases, respectively. In the 125 ppm group, the N2-ethylidene-dG levels from Aldh2+/+ mice (n=7) and Aldh2-/- mice (n=7) increased to 87.7 ± 35.0 and 69.0 ± 49.9 adducts per 108 bases, respectively. Those of Aldh2+/+ (n=10) and Aldh2-/- mice (n=10) exposed to 500 ppm further increased to 90.1 ± 36.0 and 84.6 ± 67.2 adducts per 108 bases, respectively (Figure 3). Intriguingly, the liver DNA of Aldh2-/- mice always showed decreased levels of N2-ethylidene-dG compared with that of Aldh2+/+ mice, although both Aldh2+/+ and Aldh2-/- mice showed increased levels of N2-ethylidene-dG along with the increase of acetaldehyde concentrations.

In the nasal epithelium of 125 ppm groups, the N2-ethylidene-dG levels were much higher than in other organs of both Aldh2+/+ and Aldh2-/- mice. In the nasal epithelium, the N2-ethylidene-dG level of Aldh2+/+ mice (n=3, 581 ± 61.4) was greatly increased compared with Aldh2-/- mice (n=3, 193 ± 158) (Figure 4). In the lung of 125 ppm groups, Aldh2+/+ mice showed a higher level of N'-ethylidene-dG (n=7, 68.4 ± 12.4) than Aldh2-/- mice (n=7, 54.7 ± 8.39) as shown in Figure 5; this difference is statistically significant. In 500 ppm groups, the difference between Aldh2+/+ (n=10, 283 ± 82.3) and Aldh2-/- mice (n=10, 171 ± 49.8) was greater than that observed in the 125 ppm groups. In the lung, dramatically increased levels of N'-ethylidene-dG were observed in the 500 ppm groups only, in both Aldh2+/+ and Aldh2-/- mice although it occurred in the 125 ppm groups as well in the nasal epithelium.

Compared to other organs, the level of N'-ethylidene-dG was low in the control group in the dorsal skin of both Aldh2+/+ (n=10, 26.5 ± 3.9) and Aldh2-/- mice (n=10, 28.0 ± 4.8). In the 500 ppm exposed-group, the value was increased approximately 4-fold compared with control group in Aldh2+/+ mice (n=10, 122 ± 52.4) while it was doubled in Aldh2-/- mice (n=10, 48.6 ± 16.8) (Figure 6).

5. DISCUSSION

Previously, Isse et al. showed that the blood acetaldehyde concentration of Aldh2+/+ mice (247.2 μM) was greatly higher than that of the Aldh2-/- mice (14.0 μM) one hour after the administration of ethanol by gavage at doses of 5.0 g/kg body weight (28). Matsuda et al. showed that in the liver of alcohol-fed mice (20% ethanol for 5 weeks) the adduct level of Aldh2+/+ mice (79.9 ± 14.2 adducts per 1017 bases) was much higher than Aldh2-/- (7.9 ± 1.8 adducts per 1017 bases), and indicated that the N'-ethylidene-dG level in the liver was alcohol- and Aldh2 genotype-dependent (24). In our inhalation experiments, there were no differences in the levels of N'-ethylidene-dG in the liver between Aldh2+/+ and Aldh2-/- mice (Figure 3), although the mean blood acetaldehyde concentration of Aldh2-/+ mice was more than five times as high as that of Aldh2-/- mice in the 500 ppm exposure groups. We also observed that the adduct level of Aldh2+/+ mice was rather low in the liver compared to Aldh2-/- mice, although the adduct level of Aldh2+/+ mice was always higher in other organs than that of Aldh2-/- mice. Acetaldehyde is metabolized, not only by ADH2-ALDH2 metabolic
DNA adducts in Aldh2KO mice after acetaldehyde inhalation

Figure 2. Representative chromatograms of the $N^2$-Et-dG analysis by LC/MS/MS. A and B, representative LC/MS/MS chromatograms of transition m/z 301→185 for $[^{15}N_5]$ $N^2$-Et-dG as an internal standard (24). C and D: Typical LC/MS/MS chromatograms of $N^2$-ethyl-2'-deoxyguanosine ($N^2$-Et-dG) derived from $N^2$-ethylidene-2'-deoxyguanosine ($N^2$-ethylidene-dG) in nasal epithelium DNA of Aldh2$^+/-$ (C) and Aldh2$^{+/+}$ (D) mice in 125 ppm exposure groups.

Figure 3. The $N^2$-ethylidene-dG level in the liver DNA. The levels of $N^2$-ethylidene-dG in the liver chromosomal DNA from both Aldh2$^{+/+}$ and Aldh2$^{-/-}$ mice were analyzed.
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Figure 4. The $N^2$-ethylidene-dG level in the nasal epithelium DNA. Nasal epithelium tissues were dissected from nasal cavity, using a spatula. The levels of $N^2$-ethylidene-dG in the nasal epithelium chromosomal DNA from both $Aldh2^{+/+}$ and $Aldh2^{-/-}$ mice were analyzed.

Figure 5. The $N^2$-ethylidene-dG level in the lung DNA. The levels of $N^2$-ethylidene-dG in the lung chromosomal DNA from both $Aldh2^{+/+}$ and $Aldh2^{-/-}$ mice were analyzed.
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Figure 6. The \(N^2\)-ethylidene-dG level in the dorsal skin DNA. The levels of \(N^2\)-ethylidene-dG in the dorsal skin chromosomal DNA from both Aldh2\(^{+/+}\) and Aldh2\(^{-/-}\) mice were analyzed.

Figure 7. Comparison of the \(N^2\)-ethylidene-dG level among tissues from 0 ppm exposure groups. The data from figure 3, 5, and 6 were statistically analyzed as described in “Methods and Materials”.

Pathway but also by microsomal ethanol oxidizing system (MEOS), such as cytochrome P450 (CYP) 2E1. Following alcohol drinking, the MEOS is induced, causing enhancement of metabolic activity of ethanol in the liver (22, 32). Induced MEOS in the liver, might reduce the hepatocyte acetaldehyde concentration when the blood
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Figure 8. Comparison of the $N^2$-ethylidene-dG level among tissues from 125 ppm exposure groups. The data from figure 3, 4, and 5 were statistically analyzed as described in “Methods and Materials”.

Acetaldehyde concentration is low. This might explain the difference in the results of Matsuda et al. and our present findings. Because of the requirement of metabolizing aldehyde compounds derived from food, Aldh2$^{-/-}$ mice might accumulate induced MEOS in their liver. The induced MEOS may metabolize acetaldehyde in the liver. Therefore, the adduct level can be low in the liver of Aldh2$^{+/+}$ when compared to Aldh2$^{+/+}$ mice; this was the case in the present study. However, when the mice are fed with alcohol, it is metabolized to acetaldehyde in the liver, suggesting that the liver of alcohol-fed mice might be exposed at a higher levels of acetaldehyde than in the mice exposed to air containing acetaldehyde.

In the nasal epithelium, lung, and dorsal skin, the $N^2$-ethylidene-dG levels in Aldh2$^{+/+}$ mice were higher than those of Aldh2$^{-/-}$ mice. The Aldh2 protein is expressed in the liver, lung, heart, kidney, testis, colon, and pancreas, and with less extent in esophagus and stomach (2). Our results suggest that, not only lung but also nasal epithelium and dorsal skin may express the Aldh2b protein. The local metabolism of acetaldehyde in the nasal epithelium, lung and dorsal skin, particularly the accumulation of acetaldehyde caused by Aldh2 genotype, may be directly associated with elevated $N^2$-ethylidene-dG levels.

In the liver and lung of both Aldh2$^{+/+}$ and Aldh2$^{-/-}$ mice, the $N^2$-ethylidene-dG levels of 0 ppm exposure groups were higher than that in the dorsal skin (Figure 7). The blood volume in the liver and lung is higher than that of dorsal skin. Therefore, the chromosomal DNA may be exposed to acetaldehyde derived from food more frequently in the liver and lung than in the dorsal skin; this results in elevated levels of DNA-adduct in the liver and lung compared to dorsal skin even without acetaldehyde exposure. Acetaldehyde easily dissolves in saliva, and the nasal epithelium is the first respiratory organ exposed to acetaldehyde. In the 125 ppm-exposed group, chromosomal DNA of nasal epithelium showed a high level of $N^2$-ethylidene-dG compared to those from the liver and lung (Figure 8). Similar acetaldehyde inhalation studies in rats also showed that nasal adenocarcinoma occurred in male rats exposed to 750 ppm acetaldehyde for more than 12 months (33-36). Since the lung is the second respiratory organ, chromosomal DNA in the lung is exposed to a high concentration of acetaldehyde in the inhalation experiments. The level of $N^2$-ethylidene-dG in the lung of both Aldh2$^{+/+}$ and Aldh2$^{-/-}$ mice exposed to 500 ppm acetaldehyde was higher than that in the liver and the dorsal skin (Figure 9). Our results from acetaldehyde inhalation experiments indicate that the level of DNA adduct production is in the following order: nasal epithelium > lung > liver and dorsal skin.

The Japan Society for Occupational Health (JSOH) recommends the Occupational Exposure Limits (OELs) as reference values for preventing adverse health effects on workers caused by occupational exposure to chemicals and OEL for acetaldehyde is determined as 50 ppm (37). Threshold Limit Value-Ceiling of American
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Figure 9. Comparison of the N2-ethylidene-dG level among tissues from 500 ppm exposure groups. The data from figure 3, 5, and 6 were statistically analyzed as described in “Methods and Materials

Conference of Governmental Industrial Hygienists (ACGIH) for acetaldehyde determined as 25 ppm (http://www.acgih.org/home.htm). On the other hand, the Ministry of Health, Labour and Welfare reported that the acetaldehyde amounts of the mainstream smoke and the secondhand smoke of the cigarette were 408 ± 210 and 1,710 ± 105 were µg per cigarette, respectively. The acetaldehyde concentration of the secondhand smoke of the cigarette was 2.1 – 4.6 mg/L (38). Wong et al. showed that the acetaldehyde concentration in the breath was 0.22 ± 0.10 µg/L 30 minutes after drinking ethanol (0.3 g/kg body weight) and the 1/2 elimination for acetaldehyde was 2.25 hours (39). The air containing acetaldehyde at levels of 125 ppm and 500 ppm seems to be high when it compared with a usual exposure level, such as indoor pollutions although it could happen on the labor site. Therefore, individuals with the ALDH2*2 allele should be aware of an elevated risk of the incidence of cancer in the respiratory system and skin after acetaldehyde inhalation.

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7. REFERENCE


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**Abbreviations:** ALDH2, aldehyde dehydrogenase 2; *Aldh2KO mice*, *Aldh2knockout mice*; *N*-ethylidene-dG, *N*-ethylidene-2'-deoxyguanosine; MEOS, microsomal ethanol oxidizing system; CYP, cytochrome P450; OEL, Occupational Exposure Limits.

**Key Words:** aldehyde dehydrogenase 2, *Aldh2knockout mice*, DNA adduct, *N*-ethylidene-dG

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