

Urinary 8-Hydroxydeoxyguanosine (8-OHdG) and Plasma Malondialdehyde (MDA) Levels in *Aldh2* Knock-Out Mice under Acetaldehyde Exposure

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Abstract: To clarify the carcinogenicity of acetaldehyde when associated with *ALDH* (aldehyde dehydrogenase) 2 polymorphism, *Aldh2* knock-out (*Aldh2*^{-/-}) mice and their wild type (*Aldh2*^{+/+}) mice were exposed to two different concentrations of acetaldehyde (125ppm and 500ppm) for two weeks. *Aldh2*^{-/-} mice, which have the same genetic background as C57BL/6J (wild mice) except for the *Aldh2* gene, were used as models of humans who lack ALDH2 activity. Urinary 8-hydroxydeoxyguanosine (8-OHdG) and plasma malondialdehyde (MDA) levels were measured as indicators of oxidative DNA damage and lipid peroxidation, respectively. At 125 ppm acetaldehyde exposure for 12 d, urinary 8-OHdG levels in *Aldh2*^{+/+} mice did not increase. However, urinary 8-OHdG levels in *Aldh2*^{-/-} mice were slightly increased by the end of the exposure. On the other hand, plasma MDA levels did not increase in either *Aldh2*^{-/-} or *Aldh2*^{+/+} mice. At 500 ppm, urinary 8-OHdG levels in both *Aldh2*^{-/-} and *Aldh2*^{+/+} mice significantly increased after 6 and 12 d, but there was no genetic difference. On the other hand, plasma MDA levels in *Aldh2*^{+/+} and *Aldh2*^{-/-} mice did not increase at either 125 ppm or 500 ppm after two weeks of exposure. In conclusion, it is suspected that DNA was damaged by acetaldehyde inhalation, and that susceptibility to acetaldehyde varies according to *Aldh2* genotype.

Key words: ALDH2, Polymorphism, Acetaldehyde, 8-hydroxydeoxyguanosine, Malondialdehyde, Knock-out mouse

Introduction

Acetaldehyde has recently been noted as one of the major indoor pollutants because it is used as a substitute for formaldehyde, which is used as a bonding agent of wallpaper¹. Acetaldehyde is metabolized to acetic acid by aldehyde dehydrogenase (ALDH) in humans². ALDH has

been reported to have nine isozymes (ALDH1-9)³. Among them, ALDH2, which has been called low Km ALDH, plays a major role in metabolizing acetaldehyde to acetic acid⁴. However, about half of all Japanese people lack ALDH2 activity due to a point mutation in the *ALDH2* gene⁵⁻⁸. ALDH2 deficient with a drinking habit are reported to have a higher risk of developing head and neck cancers such as esophageal, pharyngeal and oral cavity cancers compared to ALDH2 normal individuals⁹⁻¹¹.

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In order to clarify the effects of *ALDH2* polymorphism on the carcinogenicity of acetaldehyde, *Aldh2* knock-out (*Aldh2*^{-/-}) mice were used as a model of ALDH2 deficient humans. *Aldh2*^{-/-} and wild type (C57BL/6J, *Aldh2*^{+/+} mice) mice were exposed to two different concentrations of acetaldehyde (125 ppm and 500 ppm) for two weeks in an exposure chamber and their urinary 8-OHdG and plasma MDA levels were measured in order to compare DNA damage and lipid peroxidation, respectively.

Materials and Methods

Experimental animals

The *Aldh2*^{-/-} mice were generated as described previously¹². The mice were backcrossed with C57BL/6J mice for more than 10 generations. Male *Aldh2*^{+/+} and *Aldh2*^{-/-} mice aged 16 wk old were used in this study.

Acetaldehyde exposure

The *Aldh2*^{+/+} and *Aldh2*^{-/-} mice were placed in an exposure chamber (Fig. 1) and exposed to 125 ppm (225 mg/m³) or 500 ppm (900 mg/m³) of acetaldehyde for 14 d continuously. They were kept on a 12-h light (7–19 o'clock)/dark (19–7 o'clock) cycle with free access to food and water. The numbers of experimental animals were seven each for the 125 ppm exposure and ten each for the 500 ppm exposure. The acetaldehyde concentration was monitored by an acetaldehyde detector tube (GASTEC) and a DNPH absorbance tube. The details are described by Isse *et al.*¹³.

Measurement of urinary 8-hydroxydeoxyguanosine

Urine samples were collected at the same time (approximately 9:30 a.m.) on the day before, 6 d after, and 12 d after starting the exposure. Urinary 8-OHdG concentration was measured with the New 8-OHdG Check (Japan Institute for The Control of Aging). Urinary creatinine levels were also measured with the Creatinine test WAKO (Jaffé method) in order to adjust urine concentration.

Measurement of malondialdehyde (MDA) in plasma

Blood samples were collected into heparinized syringes immediately after two weeks exposure from the heart and centrifuged to collect the plasma. Plasma from control mice, that is unexposed mice, was also collected in the same way. Plasma MDA was measured with the BIOXYTECH MDA-586 kit (Oxis Research).

Statistical analyses

The changes in body weight before and after exposure, and by genotypes were analyzed by the student t-test.

Statistical analyzes of 8-OHdG and MDA levels were performed using analysis of variance (ANOVA). The difference between each group was analyzed by the Schffé method.



Fig. 1. *Aldh2*^{+/+} and *Aldh2*^{-/-} mice were placed in an exposure chamber and exposed to 125 ppm or 500 ppm acetaldehyde for 14 d continuously.

Results

The changes in body weight and activities of Aldh2^{+/+} and *Aldh2*^{-/-} mice

During 125 ppm acetaldehyde exposure for 12 d, the body weight was 27.6 ± 1.3 g before exposure compared to 27.5 ± 1.4 g after exposure, and that of *Aldh2*^{-/-} mice was 27.4 ± 1.6 g before exposure compared to 27.4 ± 1.7 g after exposure. In the case of 500 ppm acetaldehyde exposure for 12 d, the body weight of *Aldh2*^{+/+} mice was 25.4 ± 1.2 g before exposure and 24.9 ± 1.4 g after exposure, and that of *Aldh2*^{-/-} mice was 24.3 ± 1.4 g before exposure and 24.7 ± 1.3 g after exposure.

The body weights of both *Aldh2*^{+/+} and *Aldh2*^{-/-} mice were constant. Differences in body weight by *Aldh2* genotype were not recognized either.

The activities of both mice were not apparently different during exposure.

The levels of urinary 8-OHdG

Urinary 8-OHdG levels in the *Aldh2*^{+/+} mice did not change during the 125 ppm acetaldehyde exposure for 12 d (Fig. 2). In *Aldh2*^{-/-} mice, the urinary 8-OHdG level did not increase on day 6. After 12 d exposure, urinary 8-OHdG levels in *Aldh2*^{-/-} mice were about two times higher than those in *Aldh2*^{+/+} mice. However, there was no significant difference between the 8-OHdG levels of the two mouse genotypes.

For the 500 ppm acetaldehyde exposure, urinary 8-OHdG levels significantly increased during 12 d exposure in both

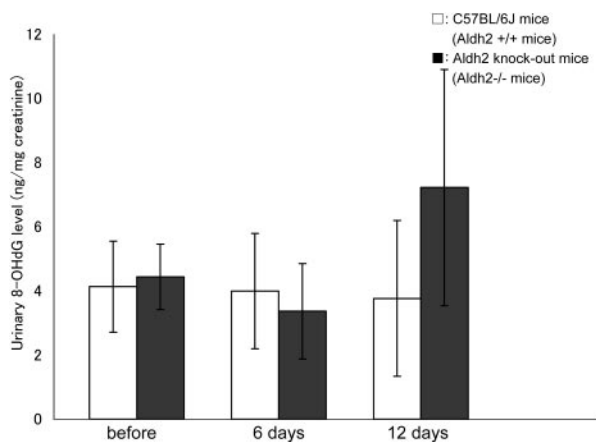


Fig. 2. Urinary 8-OHdG levels at 125 ppm acetaldehyde exposure.

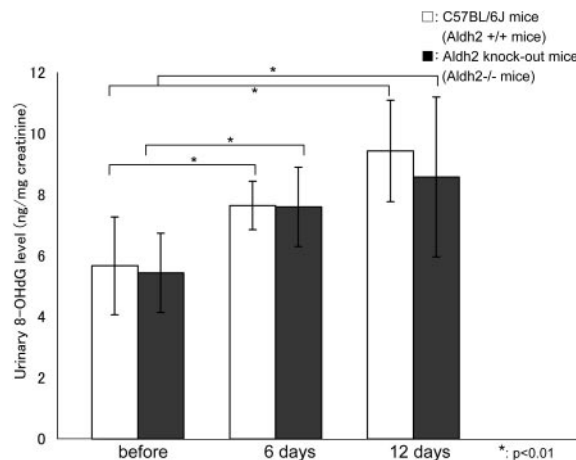


Fig. 3. Urinary 8-OHdG levels at 500 ppm acetaldehyde exposure.

Aldh2^{+/+} and *Aldh2*^{-/-} mice (Fig. 3). However, no significant differences in 8-OHdG levels by *Aldh2* genotype were observed before exposure, on day 6, or on day 12.

The levels of plasmatic MDA

Plasma MDA levels after 125ppm and 500ppm acetaldehyde exposure for two weeks are shown in Fig. 4.

Plasma MDA levels of *Aldh2*^{+/+} and *Aldh2*^{-/-} mice were compared before and after acetaldehyde exposure (125 ppm and 500 ppm). The MDA levels of both mouse groups were the same before acetaldehyde exposure. Exposure to acetaldehyde did not significantly increase plasma MDA levels in either the 125 ppm or 500 ppm exposure groups. There was no significant difference in MDA levels between *Aldh2*^{+/+} and *Aldh2*^{-/-} mice.

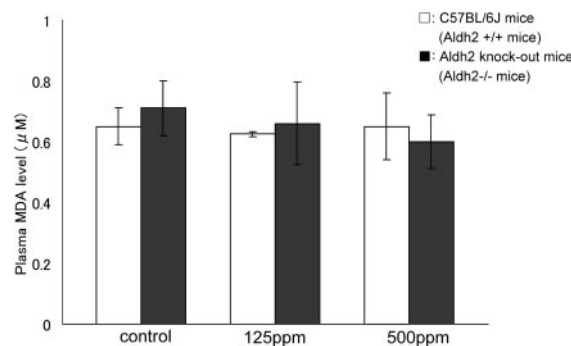


Fig. 4. Plasma MDA levels at 125 ppm and 500 ppm acetaldehyde exposure.

Discussion

The exposure concentrations used in this study, 125 ppm and 500 ppm, are based on NOEL (No Observed Effect Level) and NOAEL (No Observed Adverse Effect Level) for acetaldehyde. An acetaldehyde exposure study on rats (0, 150, and 500 ppm, 6 h a day, 5 d a week, for 4 wk) indicated that the NOEL for acetaldehyde in rats is 150 ppm¹⁴⁻¹⁶. Another exposure study on hamsters (390, 1,340, and 4,560 ppm, 6 h a day, 5 d a week, for 90 d) indicated that the NOAEL for acetaldehyde in hamsters is 390 ppm¹⁴. The exposure concentrations were decided as 125 ppm and 500 ppm in order to place them between the concentrations mentioned above.

Acetaldehyde is known to be sufficiently carcinogenic in experimental animals¹⁷. Because the mechanism of its carcinogenicity is reported to be genotoxic¹⁷, DNA damage caused by acetaldehyde was studied by measuring urinary 8-OHdG. In our study, urinary 8-OHdG levels increased at

500 ppm acetaldehyde exposure both in *Aldh2*^{+/+} and *Aldh2*^{-/-} mice. On the other hand, urinary 8-OHdG levels increased at 125 ppm acetaldehyde exposure only in *Aldh2*^{-/-} mice. It is suspected that *Aldh2*^{-/-} mice are more sensitive to acetaldehyde than *Aldh2*^{+/+} mice.

According to an IARC document, the carcinogenicity of acetaldehyde in humans is not inadequate¹⁷. On the other hand, the carcinogenicity of drinking alcohol was demonstrated in Japanese, in relation to *ALDH2* polymorphism⁹⁻¹¹. That is, the risk of esophageal cancer in subjects with one *ALDH2**2 allele (inactive type) was substantially higher in both alcoholics (odds ratio = 7.6; 95% confidence interval = 2.8–20.7) and nonalcoholic drinkers (odds ratio = 12.1; 95% confidence interval = 3.4–42.8) compared to those with *ALDH2**1/*1 (active type). Yokoyama *et al.*⁹ suspected that acetaldehyde (a recognized animal carcinogen) plays a pivotal role in the pathogenesis of alcohol-related esophageal cancer in humans because individuals with at least one *ALDH2**2 allele have a high

concentration of blood acetaldehyde after drinking alcohol. Even though our results showed no significant differences, the upward tendency of urinary 8-OHdG levels in *Aldh2*^{-/-} mice at 125 ppm exposure might be a supporting evidence for the carcinogenicity of acetaldehyde in ALDH2 deficient individuals. Further investigation will be needed to clarify this.

8-OHdG production is induced by the oxidation of deoxyguanosine (dG), which is one of the components of DNA¹⁸. Hydroxyl radicals (\cdot OH) directly act on dG to form 8-OHdG. It is stable in humans, and is excised by repair enzymes like OGG1 and excreted in urine¹⁹.

8-OHdG formation in DNA may also be related to tumorigenesis because many mutagens, tumor promoters and carcinogens are known to generate oxygen radicals, and this generation of oxygen radicals in vivo is thought to be relevant to carcinogenesis¹⁸.

Up to the present, 8-OHdG levels have been reported to increase following exposure to X-rays²⁰, ultraviolet rays²¹, chemicals such as asbestos²², benzene²³, and heavy metals such as cadmium (Cd)^{24, 25}, nickel (Ni)²⁶, and chromium (Cr)²⁷. Most of these physical and chemical factors produce ROS (\cdot OH) directly or indirectly, which damages DNA, with this being the ordinal mechanism of 8-OHdG formation.

The increase in urinary 8-OHdG levels following acetaldehyde inhalation in the present study indicates ROS production by acetaldehyde, either directly or indirectly.

MDA is used as an indicator of lipid peroxidation²⁸. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, of which the most abundant is malondialdehyde (MDA). Increased levels of lipid peroxidation products have been associated with a variety of chronic diseases in humans^{29, 30}.

MDA did not increase at either 125 ppm or 500 ppm acetaldehyde exposure in the present study, even though urinary 8-OHdG increased. The same result has been reported in a toluene inhalation experiment. Tokunaga *et al.*³¹ exposed rats to toluene (1,500 ppm, 4 h a day, for 7 d) and observed increases in 8-OHdG in the lungs, liver and kidneys, without increases in 4-hydroxy-nonenal or lipid peroxides (LPO) in these organs. They did not discuss the reason why only 8-OHdG increased, and not LPO. We suppose that this is because there is a difference between the sensitivity of 8-OHdG and LPO including MDA.

Acetaldehyde is one of the major indoor pollutants in Japan. The guideline value for the indoor air concentration of acetaldehyde was established as 0.03 ppm (48 μ l/m³). This value was arrived at following an inhalation study of acetaldehyde using rats³². In humans, some ethnic groups (e.g. Asians) lack ALDH2 activity but rats do not have *Aldh2* polymorphism. When the guideline value was made by extrapolating the animal experiment to humans, *ALDH2*

polymorphism was not considered.

We suspect from the present study that individuals who lack ALDH2 activity are more sensitive to acetaldehyde than those with normal ALDH2 activity, especially around 125 ppm. However there may be some limitations in extrapolating this result to very low exposure concentrations of around 0.03 ppm. Further studies are needed to confirm whether this guideline value for the indoor air concentration is suitable for individuals who lack ALDH2 activity.

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