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 wallpaper. Acetaldehyde is metabolized to acetic acid by aldehyde dehydrogenase (ALDH) in humans. ALDH has been reported to have nine isozymes (ALDH1-9). Among them, ALDH2, which has been called low Km ALDH, plays a major role in metabolizing acetaldehyde to acetic acid. However, about half of all Japanese people lack ALDH2 activity due to a point mutation in the ALDH2 gene. 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 individuals.

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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 previously\(^{12}\). 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/m\(^3\)) or 500 ppm (900 mg/m\(^3\)) 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 analyses of 8-OHdG and MDA levels were performed using analysis of variance (ANOVA). The difference between each group was analyzed by the Schffé method.
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.

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 ppm4-16. 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 ppm19. 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 polymorphism8-11. 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 DNA[39]. Hydroxyl radicals (·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 urine[19].

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 carcinogenesis[18].

Up to the present, 8-OHdG levels have been reported to increase following exposure to X-rays[20], ultraviolet rays[21], chemicals such as asbestos[22], benzene[23], 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 (·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 peroxidation[28]. 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 µl/m³). This value was arrived at following an inhalation study of acetaldehyde using rats[32]. 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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