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Susceptibility to inhalation toxicity of acetaldehyde in *Aldh2* knockout mice

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1. ABSTRACT

In this study, we evaluated the inhalation toxicity of acetaldehyde in *Aldh2* KO (*Aldh -/-*) mice, using pathological method. Male C57BL/6 (*Aldh2* +/+) mice and *Aldh -/-* mice were exposed to atmospheres containing acetaldehyde at levels of 0, 125, and 500 ppm for 24 h/day during 14 days. Although the average blood acetaldehyde concentration of *Aldh -/-* mice was higher than that of *Aldh2 +/+* mice in the acetaldehyde exposure group, observable effects by the acetaldehyde exposure on the lung and liver were not different between wild type and ALDH2 null mice. In *Aldh2-/-* mice, the levels of 1) erosion of respiratory epithelium and the subepithelial hemorrhage in nose, 2) hemorrhage in nasal cavity, 3) degeneration of respiratory epithelium in larynx, pharynx and trachea, and 4) degeneration of dorsal skin were higher compared with *Aldh2 +/+* mice, indicating that *Aldh2-/-* mice are more acetaldehyde-sensitive than *Aldh2+/+* mice. This is the first example for studying pathological effects of Aldh2 deficiency using *Aldh -/-* mice exposed to a low level of acetaldehyde.

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 causes harmful consequences for many organs, which is associated with the incidence of various cancers, such as esophageal cancer (3-5). Many epidemiological studies show 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 variations among individuals are of great interest for the 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). *ALDH2*2*, being a genetic polymorphism of ALDH2

and having an amino acid substitution from glutamic acid at 487 to lysine (E487K), is widely prevalent in some Asian populations (11). ALDH2 functions as a homotetramer, and the inactive subunit produced by the *ALDH2*2* allele also acts in a dominant negative fashion. Therefore, individuals with the *ALDH2*2* allele show high blood acetaldehyde concentrations after the intake of only a moderate amount of alcohol (2). As consequence of the decreased acetaldehyde metabolism, the *ALDH2*2* allele is associated with alcohol-induced flushing, and is also positively related to liver disease, oral cancer and esophageal cancer (5), while it negatively affects coronary heart disease (2).

Aldh2 knockout (KO) mice have already been generated in our laboratory (12). These mice (C57BL/6), lacking *Aldh2*, should be a useful animal model to investigate the effects of Aldh2 deficiency (2). Since susceptibility to inhalation toxicity of acetaldehyde is still unclear in individuals with the *ALDH2*2* allele, in this study, we evaluated the inhalation toxicity of acetaldehyde in *Aldh2* KO (*Aldh2* -/-) mice, using pathological method.

3. MATERIALS AND METHODS

3.1. Wild mice (*Aldh2* +/+) and *Aldh2* KO mice (*Aldh2* -/-)

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 (12). *Aldh* -/- mice were backcrossed with a C57BL/6 strain for more than 10 generation.

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. The mice cages, floor beds, and rodent chow were used after autoclaving. The mice cage was cleaned every day. All the mice were treated in accordance with the guidelines of the Animal Welfar and Ethics Committee of the Animal Care and Experimentation of the UOEH (13-15).

3.2. Treatments

Ten of each *Aldh2* +/+ and *Aldh2* -/- mouse was exposed to filtered atmospheric air (0 ppm) as controls. Seven of each *Aldh2* +/+ and *Aldh2* -/- mice were exposed to the air containing acetaldehyde at levels of 125 ppm (125 ppm exposure group). Seven of each *Aldh2* +/+ and *Aldh2* -/- mice were exposed to the air containing acetaldehyde at levels of 500 ppm (500 ppm exposure group). Mice were divided at random. Acetaldehyde level in cage was evaluated every 6 hours by acetaldehyde detector tubes (Gastec corp., Kanagawa, Japan) and Sep-Pak DNPH-Silica (Waters corp., MA, USA) (14). Mice were exposed to atmospheres containing acetaldehyde for 24 h/day during 14 days. During this period body weights were recorded every day and at the end of the observation period mice were sacrificed.

3.3. Blood acetaldehyde concentration

Mouse blood was collected from the decapitated trunk into liquid nitrogen-cooled plastic tubes and stored. The blood was transferred into ice cold 0.6 N perchloric acid solution (PCA) and centrifuged. Sample (0.5 mL) was collected and transferred to gas-tight vials with caps. Acetaldehyde concentration was measured as a previously described (13), using a Hewlett-Packard headspace sampler (HP7694; Wilmington, DE), Hewlett-Packard gas chromatograph (HP6890, Wlmington, DE) connected to a mass spectrometer (JOEL JMS-BU20, Tokyo, Japan), and a 60 m x 0.25 mm inner diameter AQUATIC capillary column (GL Sciences, Tokyo, Japan) with a film thickness of 1.0 μm.

3.4. Staining

At the end of the treatment period mice were sacrificed by bleeding under ether anesthetization and examined for gross pathological changes. Samples of the organs, such as nose, larynx, pharynx, trachea, lung, liver, auricle, and dossal skin, were preserved in a 4% neutral aqueous phosphate-buffered formaldehyde solution. Following fixation the heads were decalcified for 48 hours in Panapharm Laboratories Co., Ltd. (Kumamoto, Japan). Three transverse sections across the nose were made to investigate the nasal epithelium, nasal cavity and paranasal sinuses (Figure 1) (16). Distribution of three kinds of nasal epithelium (Squamous epithelium, respiratory epithelium and olfactory epithelium) was also shown in Figure 1. Larynx, pharynx, and trachea were investigated by the maximum sagittal sections (Figure 2). Lung, liver, auricle, and dossal skin were investigated by the maximum sections. Samples of the organs were embedded in paraffin and sectioned at 4 μm and stained with hematoxylin and eosin.

3.5. Statistics

Analysis of co-variance was carried out on the body weight. For histochemical changes, the chi-square test was used.

4. RESULTS

4.1. Blood acetaldehyde concentration and body weight

The average actual exposure concentrations of 125 ppm exposure group and 500 ppm exposure group were 126.3 ppm and 510.5ppm, respectively. The mean blood acetaldehyde concentration of *Aldh2* +/+ mice (n=3) and *Aldh2* -/- mice (n=3) in 125 ppm exposure group were 1.65 μM and 2.39 μM. The mean blood acetaldehyde concentration of *Aldh2* +/+ mice (n=3) and *Aldh2* -/- mice (n=3) in the 500 ppm exposure group were 1.72 μM and 8.90 μM. 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 group. The mean mice body weights were shown in Table 1. The mean mice body weight of the 500 ppm exposure group after treatment was significantly lower than that of the control group after treatment and the 500 ppm exposure group before treatment ($p < 0.01$).

Table 1. The mean mice body weights in the various groups

		Aldh2 +/+ mice			*Aldh* -/- mice		
Body weight (g)		**n**	**mean (μM)**	**SD**	**n**	**Mean (μM)**	**SD**
Control group	Before treatment	10	27.1	1.25	10	26.5	1.66
	After treatment	10	28.2	1.37	10	27.9	1.73
125 ppm exposure group[1]	Before treatment	7	27.8	1.33	7	27.4	1.61
	After treatment	7	27.4	1.32	7	27.7	1.75
500 ppm exposure group[2]	Before treatment	10	26.6	1.79	10	27.1	1.41
	After treatment[3]	10	21.8	1.21	10	23.9	1.23

[1]Exposed to atmospheres containing acetaldehyde at levels of 125 ppm, [2]Exposed to atmospheres containing acetaldehyde at levels of 500 ppm, [3]Comparing with control group after treatment and 500 ppm exposure group before treatment ($p<0.01$)

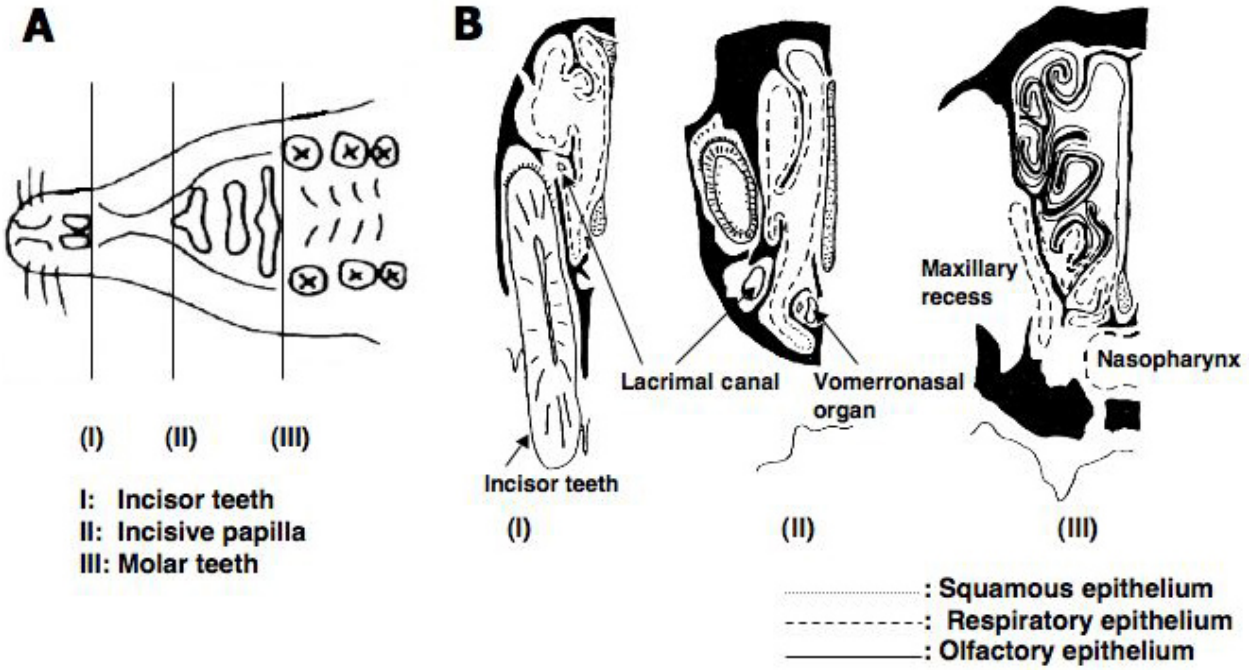

Figure 1. Three transverse sections across the nose. A, Vertical view of the section levels for microscopic examination (Level I; incisor teeth, Level II; incisive papilla, Level III; molar teeth). B, Distribution of three kinds of nasal epithelium (Squamous epithelium, respiratory epithelium and olfactory epithelium).

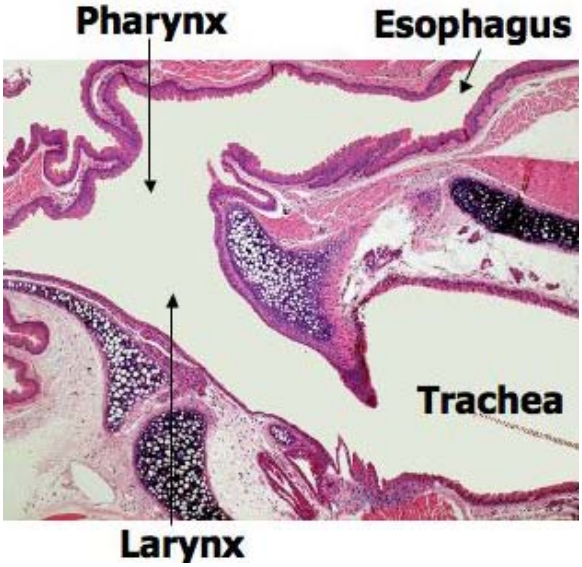

Figure 2. The maximum sagittal sections made to investigate larynx, pharynx, and trachea. Larynx, pharynx, and esophagus were covered by squamous epithelium and trachea by respiratory epithelium.

Table 2. Histopathological changes and number of mice showing the lesions in the various groups[1]

	Aldh2 +/+[2]			*Aldh2 -/-*[3]		
Site and type of lesions[4]		**Acetaldehyde exposure**			**Acetaldehyde exposure**	
Groups	**Control**	**125 ppm**	**500 ppm**	**Control**	**125 ppm**	**500 ppm**
I. Nose (n)	(5)	(4)	(5)	(5)	(4)	(5)
A. Squamous epithelium						
Hyperkeratinization[5]	0	0	*4 (80%)*	0	*1 (25%)*	*5 (100%)*
Broadness to respiratory epithelium	0	0	0	0	0	0
Erosion	0	0	0	0	0	0
Degeneration (atrophy, disarrangement)	0	0	0	0	0	0
Hyperplasia	0	0	0	0	0	0
B. Respiratory epithelium						
Broadness to olfactory epithelium	0	0	0	0	0	0
Erosion[6]	0	**1 (25%)**	**1 (20%)**	0	0	**5 (100%) (Ulcer;1)**
Degeneration (atrophy, disarrangement)[5]	0	*2 (50%)*	*3 (60%)*	0	*3 (75%)*	*4 (80%)*
Slight		*0*	*1*		*0*	*0*
Moderate		*2*	*2*		*3*	*2*
Severe		*0*	*0*		*0*	*2*
Hyperplasia	0	0	0	0	0	0
Squamous cell metaplasia	0	0	0	0	0	0
Goblet cell metaplasia	0	0	0	0	0	0
C. Olfactory epithelium						
Erosion	0	0	0	0	0	0
Degeneration (atrophy, disarrangement)[5]	0	0	*1 (20%)*	0	0	*1 (20%)*
Slight			*1*			*1*
Moderate			*0*			*0*
Severe			*0*			*0*
Hyperplasia	0	0	0	0	0	0
Metaplasia to squamous epithelium	0	0	0	0	0	0
Metaplasia to respiratory epithelium	0	0	0	0	0	0
D. Subepithelium						
Hemorrhage[6]	0	0	0	0	**2 (50%)**	**4 (80%)**
Teleangiectasia	0	0	0	0	0	0
Infiltrate of inflammatory cells	0	0	0	0	0	0
Edema	0	0	0	0	0	0
II. Nasal cavity	(5)	(4)	(5)	(5)	(4)	(5)
Hemorrhage[6]	0	0	0	0	**1 (25%)**	**1 (20%)**
Exudate[5]	0	0	*4 (80%)*	0	0	*5 (100%)*
III. Paranasal sinuses	(5)	(4)	(5)	(5)	(4)	(5)
Sinusitis[4]	**0**	**0**	**1 (20%) (Hemorrhage;1)**	**0**	**0**	**0**
IV. Larynx, pharynx and trachea	(7)	(4)	(7)	(9)	(4)	(9)
A. Respiratory epithelium						
Erosion[4]	**1 (14%)**	**4 (100%)**	**1 (14%)**	**2 (22%)**	**3 (75%)**	**3 (33%)**
Degeneration (atrophy,disarrangement)[5, 6]	0	*3 (75%)*	0	0	*3 (75%)*	**4 (44%)**
Slight		*1*			*2*	**2**
Moderate		*2*			*1*	**2**
Severe		*0*			*0*	**0**
Hyperplasia	0	0	0	0	0	0
Squamous cell metaplasia	0	0	0	0	0	0
Goblet cell metaplasia	0	0	0	0	0	0
B. Subepithelium						
Hemorrhage	0	0	0	0	0	0
Teleangiectasia	0	0	0	0	0	0
Infiltrate of inflammatory cells	0	0	0	0	0	0
Edema	0	0	0	0	0	0
V. Tracheal cavity	(7)	(4)	(7)	(9)	(4)	(9)
Hemorrhage[4]	**0**	**0**	**1 (14%)**	**2 (22%)**	**0**	**1 (11%)**
VI. Lung	(10)	(4)	(10)	(9)	(4)	(10)
A. Bronchus						
Hemorrhage in bronchus[4]	**1 (10%)**	**0**	**0**	**1 (11%)**	**0**	**0**
Erosion	0	0	0	0	0	0
Degeneration (atrophy, disarrangement)	0	0	0	0	0	0
Hyperplasia	0	0	0	0	0	0
Squamous cell metaplasia	0	0	0	0	0	0
Goblet cell metaplasia	0	0	0	0	0	0
B. Pulmonary parenchyma						
Alveolar hemorrhage[4]	**2 (20%)**	**0**	**4 (40%)**	**1 (11%)**	**1 (25%)**	**2 (20%)**
Interstitial thickness	0	0	0	0	0	0
Peribronchial changes	0	0	0	0	0	0
Hemorrhage						
Edema						
Infiltrate of inflammatory cells						

VII. Liver	(10)	(4)	(10)	(10)	(4)	(10)
Changes near central vein Hemorrhage Inflammatory cell nest Degeneration Focal necrosis	0	0	0	0	0	0
Changes near interlobular vessels[5] Hemorrhage Inflammatory cell nest Degeneration Focal necrosis	1 (10%) 1 0	*2 (50%)* *2* *0*	*6 (60%)* *6* *0*	2 (20%) 2 0	*2 (50%)* *2* *0*	*6 (60%)* *5* *1*
VIII. Auricle	(10)	(0)	(10)	(10)	(0)	(9)
A. Squamous epithelium						
Hyperkeratinization[5, 7]	0		*10 (100%)*	*5 (50%)*		*8 (89%)*
Degeneration (atrophy, disarrangement)	0		0	0		0
Hyperplasia	0		0	0		0
B. Subepithelium						
Hemorrhage	0		0	0		0
Teleangiectasia	0		0	0		0
Infiltrate of inflammatory cells	0		0	0		0
Edema	0		0	0		0
IX. Dorsal skin	(10)	(0)	(10)	(10)	(0)	(9)
A. Squamous epithelium						
Hyperkeratinization	0		0	0		0
Degeneration (atrophy, disarrangement)[6] Slight Moderate Severe	0		0	0		**7 (78%)** **3** **4** **0**
Hyperplasia	0		0	0		0
B. Subepithelium						
Hemorrhage	0		0	0		0
Teleangiectasia	0		0	0		0
Infiltrate of inflammatory cells	0		0	0		0
Edema	0		0	0		0

[1]The numbers of mice examined is given in round brackets, [2]Wild mice (C57BL/6), [3]*Aldh* knock out (*Aldh* -/-) mice, [4]Alterations by the scarification and fixation of mice (light shadow boxes written in bolds), [5]Alterations due to acetaldehyde exposure (light shadow boxes written in italics), [6]Alterations with *Aldh2-/-* are more sensible than that with *Aldh2+/+* (dark shadow boxes written in bolds), [7]Alterations with *Aldh2-/-* are more sensible than that with *Aldh2+/+* (dark shadow boxes written in italics).

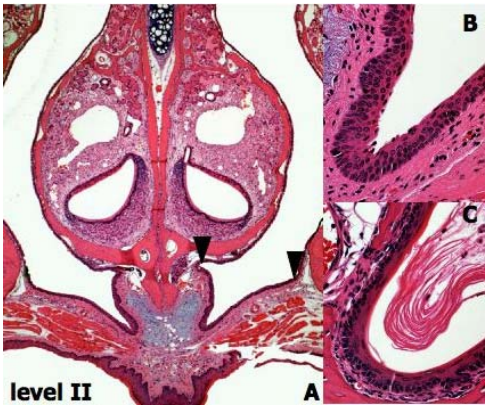

Figure 3. Hyperkeratinization of squamous epithelium in nose occurred due to acetaldehyde exposure. A, Vertical view of the section at the incisive papilla (Level II). Allows showed the range of squamous epithelium. B, Scale up the squamous epithelium of *Aldh2* +/+ control group. C, Scale up the squamous epithelium of *Aldh* -/- 500 ppm exposure group.

4.2. Pathology

Type, site, and incidence of the histopathological changes observed are shown in Table 2. Alterations by the scarification and fixation of mice were seen as hemorrhage of paranasal sinus in nose, erosion of respiratory epithelium in larynx, pharynx, and trachea, hemorrhage of bronchus, and alveolar hemorrhage in pulmonary parenchyma. These alterations are seen in most of the groups or one of the groups at random.

4.2.1. Nose

The nose was most severely affected by the acetaldehyde exposure. Hyperkeratinization in the squamous epithelium (Figure 3C) and degeneration of respiratory epithelium (Figure 4C and D) and olfactory epithelium (Figure 4F) were observed in both wild type and ALDH2 null mice.

Erosion of respiratory epithelium and the subepithelial hemorrhage was shown in Figure 5. The erosion of respiratory epithelium and the subepithelial hemorrhage were seen in *Aldh2-/-* mice exposed by acetaldehyde but not in *Aldh2+/+* mice. The rate of the erosion of respiratory epithelium in *Aldh2 -/-* acetaldehyde exposure group (55.6%; 5/9) seemed to be higher than that in *Aldh2 +/+* acetaldehyde exposure group (22.2%; 2/9). The rate of the subepithelial hemorrhage in *Aldh2 -/-* acetaldehyde exposure group (66.7%; 6/9) was significantly higher than that in *Aldh2 +/+* acetaldehyde exposure group (0%; 0/9) ($p < 0.05$).

4.2.2. Nasal cavity

The exudate caused by the acetaldehyde exposure was observed in 500 ppm exposure groups of both wild

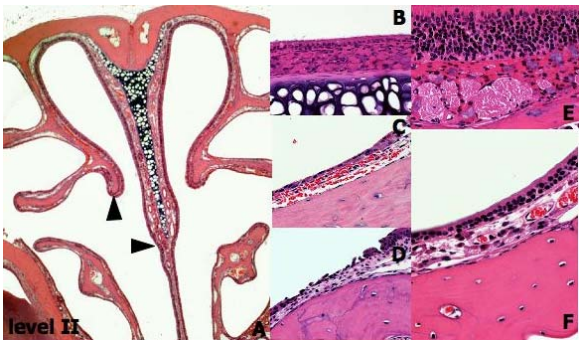

Figure 4. Degeneration of respiratory epithelium and olfactory epithelium in nose occurred due to acetaldehyde exposure. A, Vertical view of the section at the incisive papilla (Level II). Allows showed the demarcation between squamous epithelium and olfactory epithelium. B, Scale up the respiratory epithelium of *Aldh2* +/+ control group. There were columnar epithelium and goblet cells. C, Scale up the squamous epithelium of *Aldh2* +/+ 500 ppm exposure group. The loss of microvilli and thinning of the epithelium were determined as slightly degeneration. D, Scale up the squamous epithelium of *Aldh* -/- 125 ppm exposure group. Severe disarrangement and atrophy of the epithelium were determined as severe degeneration. E, Scale up the olfactory epithelium of *Aldh* -/- 500 ppm exposure group. The olfactory epithelium was usually composed of more than seven layers of olfactory cells. F, The thinning of the olfactory epithelium was determined as slightly degeneration. Hyperkeratinization of squamous epithelium occurred due to acetaldehyde exposure.

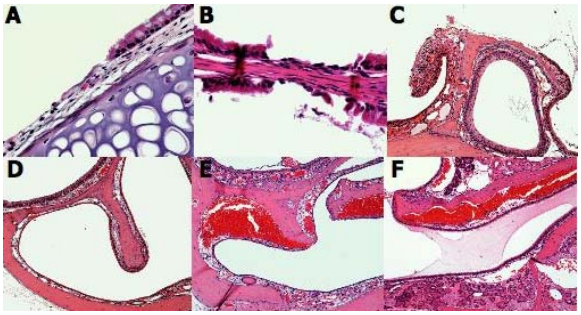

Figure 5. Erosion of respiratory epithelium and the subepithelial hemorrhage of *Aldh2*-/- mice were more sensible than that of *Aldh2*+/+ mice with acetaldehyde exposure. A Erosion of respiratory epithelium in *Aldh2*+/+ 125 ppm exposure group. B Erosion of nasal septum in *Aldh2*-/- 500 ppm exposure group. C Ulcer of turbinate in *Aldh2*-/- 500 ppm exposure group. D Vertical view of the section at the incisor teeth (Level I) in *Aldh2*+/+ control group. E Subepithelial hemorrhage in *Aldh2*-/- 500 ppm exposure group. F Subepithelial hemorrhage with exudate of nasal cavity Subepithelial hemorrhage.

type and ALDH2 null mice, and the hemorrhage was seen only in 125 and 500 ppm exposure groups of *Aldh2* -/- mice.

4.2.3. Larynx, pharynx and trachea

Degeneration of respiratory epithelium by the acetaldehyde exposure was observed (Figure 6). The rate of the degeneration of respiratory epithelium in *Aldh2* -/- acetaldehyde exposure group (53.8%; 7/13) seemed to be higher than that in *Aldh2* +/+ acetaldehyde exposure group (27.3%; 3/11).

4.2.4. Liver

The inflammatory cell nest observed in both wild type and ALDH2 null mice was increased by the acetaldehyde exposure (Figure 7).

4.2.5. Auricle and dorsal skin

Alterations due to the acetaldehyde exposure were seen as the hyperkeratinization of auricle (Figure 8B). The hyperkeratinization was observed in both control and exposed groups of *Aldh2* -/-. The rate of the hyperkeratinization of auricle in *Aldh2* -/- control group (50.0%; 5/10) was significantly higher than that of *Aldh2* +/+ control group (0.0%; 0/10) ($p < 0.05$).

Degeneration of dorsal skin was observed only in the 500 ppm exposure group of *Aldh2* -/- (as shown in Figure 8D). The rate of the degeneration of dorsal skin in *Aldh2* -/- 500 ppm exposure group (77.8%; 7/9) was significantly higher than that in *Aldh2* +/+ 500 ppm exposure group (0.0%; 0/10).

5. DISCUSSION

In this acetaldehyde inhalation study, the mean blood acetaldehyde concentration of *Aldh2* -/- mice was also significantly higher than that of *Aldh2* +/+ mice in the exposure group, which is consistent with our previous results from the ethanol gavage study of the knockout mice (13). The major sites harmed by acetaldehyde inhalation centered in epithelium tissues from the nose to trachea but not in the lung. This suggests that acetaldehyde is mostly absorbed upstream of the lung in our experimental condition. The respiratory epithelium and subepithelium in the nose and squamous epithelium in the dorsal skin showed clear differences in damages between wild type and knockout mice, suggesting that in these tissues a certain level of ALDH2 that is enough for detoxifying acetaldehyde is expressed or induced. However, in the liver, inflammatory cell nests were similarly observed near interlobular vessels in both wild type and knockout mice, suggesting that a low level of ALDH2 is expressed or that other enzymes may metabolize acetaldehyde in these tissues.

Previously, we also reported that urinary 8-hydroxydeoxyguanosine (8-OHdG) levels in *Aldh2* -/- mice exposed to 125 ppm concentrations of acetaldehyde for two weeks were slightly increased by the end of the exposure but not in *Aldh2* +/+ mice (17), suggesting that a DNA damage and susceptibility to acetaldehyde may be increased by the deficiency of *Aldh2*. The results were supported by similar inhalation studies with acetaldehyde in rats showing that nasal adenocarcinoma occurred in male

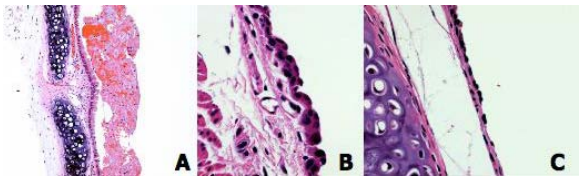

Figure 6. Degeneration of respiratory epithelium in larynx, pharynx and trachea occurred due to acetaldehyde exposure. The degeneration of respiratory epithelium of *Aldh2-/-* mice was also more sensible than that of *Aldh2+/+* mice with acetaldehyde exposure. A, Tracheal hemorrhage and respiratory epithelium. B, Scale up the respiratory epithelium of *Aldh -/-* 500 ppm exposure group. The loss of microvilli, and thinning and disarrangement of the epithelium were determined as moderately degeneration. C, Scale up the respiratory epithelium of *Aldh -/-* 125 ppm exposure group. Disarrangement and atrophy of the epithelium were determined as severe degeneration.

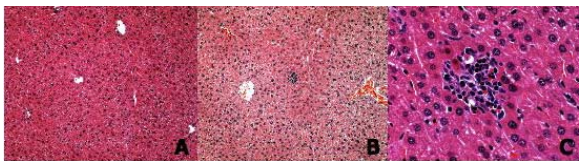

Figure 7. Inflammatory cell nests in the liver occurred due to acetaldehyde exposure. A, A representative image in the liver of *Aldh2 +/+* control group. B and C, The inflammatory cell nests of 500 ppm exposure group.

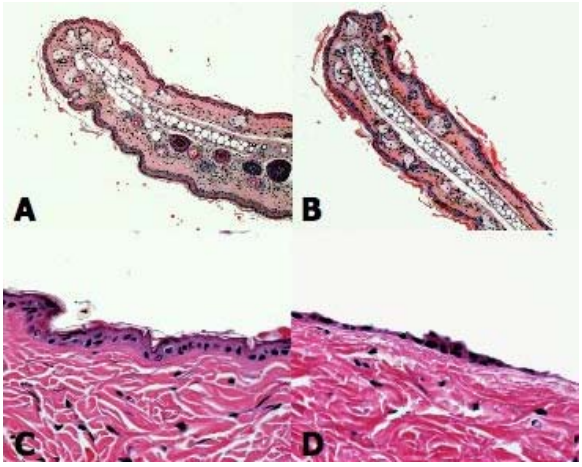

Figure 8. Hyperkeratinization of auricle (A and B) and degeneration of dorsal skin (C and D) occurred due to *Aldh2* genotype and acetaldehyde exposure. A, Auricle of *Aldh2 +/+* control group. B, The hyperkeratinization of auricle of *Aldh2 +/+* 500 ppm exposure group. C, Dorsal skin of *Aldh2 +/+* control group. D, The Degeneration of dorsal skin of *Aldh2-/-* 500 ppm exposure group. The thinning and disarrangement of the epithelium were determined as moderately degeneration.

rats exposed to 750 ppm acetaldehyde for more than 12 months (16, 18-20).

In humans, there exists a group of individuals who report a variety of symptoms on exposure to low levels of common volatile organic mixtures such as perfume, cigarette smoke, and cleaning agents. Some of these individuals report having occupied "sick buildings" during the time their symptoms began (21). There are the emerging events that are assuming increasing relevance as work-related respiratory diseases (indoor air pollution and sick building syndrome, respiratory toxicity of formaldehyde, pollutant-induced asthma, dental technician lung diseases, lung cancer from diesel exhaust, environmental silicosis) (22). The baseline prevalence of nasal symptoms among building occupants is often 20%, but in some studies it is as high as 50 to 60%. Acetaldehyde is well-known as an indoor air pollutant. Alcohol-induced bronchial asthma showed the patient as homozygous for a mutation of *ALDH2* gene in previous case report (23), suggesting that acetaldehyde produced from ethanol in the body may cause this symptom. Although the relationship between symptom and pathological changes in humans is not clear, our present study suggests the importance of understanding pathological effects of acetaldehyde by *Aldh2* genotype.

6. CONCLUSIONS

In this study, the mean blood acetaldehyde concentration of *Aldh2 -/-* mice was higher than that of *Aldh2 +/+* mice in the acetaldehyde exposure groups. We found specific alternations by the acetaldehyde exposure as follows: 1) Erosion of respiratory epithelium and subepithelial hemorrhage in the nose. 2) Hemorrhage in nasal cavity. 3) Degeneration of respiratory epithelium in larynx, pharynx, and trachea. 4) Degeneration of dorsal skin. These alternations (from 1) to 4)) were considered to be more sensible in *Aldh2 -/-* mice than in *Aldh2 +/+* mice. We also found that hyperkeratinization of auricle was induced in *Aldh2-/-* mice without acetaldehyde exposure.

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