Effects of low dose of ethanol on the senescence score, brain function and gene expression in senescence-accelerated mice 8 (SAMP8)

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Abstract. Accumulating epidemiological evidence suggests light to moderate alcohol intake reduces risk of several chronic diseases. However, there is limited information regarding the effects of low alcohol intake in animal studies. This study investigated the effect of low ethanol dosage on senescence-accelerated mouse (SAMP8), an animal model of aging and neurodegenaration. Male SAMP8 mice (11 weeks old) had free access to a commercial stock diet with drinking water containing 0, 1 or 2% (v/v) ethanol for 15 weeks. The total grading score of senescence in the 1%-ethanol group was, in large part, the lowest among the three groups. Analysis using the open-field test revealed a significant elevation (+77%, P<0.05) in the rearing activity (index of seeking behavior) in the 1%-ethanol group, but not in the 2%-ethanol group. In addition, 2% ethanol elevated spontaneous locomotor activity (+75%, P<0.05), whereas 1% ethanol did not. Scrutiny of serum parameters indicated intake of 1% ethanol significantly decreased serum insulin levels (-13%, P<0.05), whereas 2% did not. Intake of 2% ethanol significantly elevated (2.5-fold, P<0.05) *S100a8* mRNA (an inflammatory signal) in the brain, but that of 1% ethanol did not. Intriguingly, 1% ethanol intake remarkably elevated (10-fold, P<0.05) mRNA of brain alcohol dehydrogenase 1 (*Adh1*), which metabolizes lipid-peroxidation products and is involved in the synthesis of retinoic acid, a neuroprotective factor. Of note, 2%-ethanol intake did not exert this effect. Taken together, intake of 1% ethanol is likely to be beneficial for SAMP8 mice.

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Introduction

Several epidemiological studies show that all-cause mortality as well as the incidences of cardiovascular diseases, diabetes, liver cirrhosis and stroke are lower in people reporting moderate alcohol consumption than both non-drinkers and heavy drinkers; this suggests a J-shaped or U-shaped effect of alcohol consumption on human health (1-3). Recent epidemiological evidence has further suggested low or moderate intake of alcohol decreases the risk of brain diseases such as dementia and cognitive impairment (4,5). In epidemiological studies, however, it is difficult to completely adjust for confounding factors (e.g., ethnicity, beverage type, drinking style, socioeconomic status, lifestyle, physical activity and personality type) (6,7). Thus, epidemiological studies have limited power to conclude that moderate alcohol intake itself directly improves human health and exerts a biological effect. Animal experiments are useful for examining the direct effects of pure alcohol. However, experimental animal models have focused on high toxicological doses with forced and excessive ingestion (e.g., intragastric ethanol infusion and liquid diets) (8,9). Meanwhile, animal studies involving low alcohol intake are limited.

Research with experimental rodent models and cultured cardiac myocytes, or endothelial cells indicates that moderate alcohol exposure can promote anti-inflammatory processes involving adenosine receptors, protein kinase C (PKC), nitric oxide synthase, heat shock proteins, and others which could underlie cardioprotection (10). Decreased risks of cognitive loss or dementia in moderate, non-binge consumers of alcohol (wine, beer, liquor) have been reported, whereas increased risk has been reported only in a few studies (11). Thus, moderate alcohol exposure appears to trigger analogous mild stress-associated, anti-inflammatory mechanisms in the heart, vasculature, and brain that tend to promote cellular survival pathways (10). One study indicated that ethanol intake levels achieved by alcohol-preferring P rats as a result of chronic voluntary exposure may have favorable rather than detrimental effects on lipid profiles in this genetic line, consistent with data supporting beneficial cardioprotective and neuroprotective effects of moderate ethanol consumption (12). Our recent study has suggested that intake of 1% ethanol in drinking

water improved liver function in rats maintained on a high-fat diet, but that of 2% ethanol did so to a lesser extent (13). In the present study, we examined the effect of low ethanol intake on senescence in senescence-accelerated mice (SAM). SAM are widely used as an animal genetic model for studying aging, and a techniques for evaluation of senescence degree are well established (14). The system was designed to represent changes in both behavior and appearance of these mice, which display the clinical manifestations and gross lesions associated with the aging process. The defined grading score system is one of the significant advantages in aging studies using SAM. The Senescence-Accelerated Mouse Prone 8 (SAMP8) line has further advantages, because some behavioral traits and histopathology resemble human dementia as well as its recapitulating rapid physiological senescence (15,16). Thus, the present study was conducted to examine the effects of low dose of ethanol on SAMP8 mice.

Materials and methods

Animal experiment. Eight-week-old male SAMP8 mice (Japan SLC, Shizuoka, Japan) were maintained under controlled conditions (ambient temperature, 22°C ± 2°C, 12-h light/dark cycle, lights on from 12:00 a.m. to 12:00 p.m., lights off from 12:00 p.m. to 12:00 a.m.). The animals were housed individually in plastic cages (125x200x110 mm) with free access to food (MF, Oriental Yeast, Tokyo, Japan) and water. This study was approved by the Animal Care Committee of the National Research Institute of Brewing, Japan (Ethical approval No. 25-1). After a 3-week acclimation period, the mice received deionized drinking water with 0, 1% (v/v) or 2% (v/v) ethanol (n=8 mice per group) for 15 weeks. The ethanol-consuming groups had free access to only 1 or 2% ethanol without other water being available. Licking counts of drinking water were evaluated by drinking sensors (DS-1, Shinfactory, Fukuoka, Japan) for 21 h (11:00 a.m. to 08:00 a.m.) in 20-week-old mice. Food intake was quantified using measuring the difference between the preweighed pellet in food cups and the weight of remaining pellet and spill at the end of 24-h period. Fluid intake was also determined by measuring the difference between preweighed water bottle and the weight of remaining bottle at the end of 24-h period. At the termination of experimental procedure, mice were sacrificed by decapitation under diethylether anesthesia (between 01:00 p.m. and 03:00 p.m.) after removal of food and drinking water (08:00 a.m.).

Grading of senescence. The degree of senescence was evaluated by a grading system (14) comprising the following 11 items in four categories: Behaviors (reactivity and passivity), skin and hair (glossiness, coarseness and hair loss), eyes (ulcer, periophthalmic lesions, cataract, corneal ulcer and corneal opacity) and skeleton (lordokyphosis). The grading score was calculated by summing the scores of all 11 items from 0 to 4.

Serum biochemical analysis. The activities of serum alanine aminotransferase (ALT, EC 1.1.1.27) and aspartate aminotransferase (AST, EC 2.6.1.1) as well as levels of serum glucose, triglyceride, albumin, and total cholesterol were measured calorimetrically by the DRICHEM commercial assay system (Fujifilm, Tokyo, Japan). Serum insulin (Mercodia, Uppsala, Sweden), adiponectin and IGF-1 (both from R&D Systems, Minneapolis, MN, USA) were measured by commercial ELISA kits. Serum IL-1β, IL-12, and TNF-α were determined by the Bio-Plex cytokine assay kit in combination with the Bio-Plex Manager software (Bio-Rad, Hercules, CA, USA).

Open-field test. Open-field test was performed using a two-level infrared beam apparatus (Scanet MV-40; Melquest, Toyama, Japan), an automatic analysis system for measuring murine locomotor activity (17). Testing was performed between 01:00 p.m. and 03:00 p.m. Mice were placed into the center of the open field (44x44x30 cm) and left to explore for 10 min. Food and water were available *ad libitum* other than during 10-min trials. Rearing counts were evaluated as vertical activity. The field was cleaned after each session.

Spontaneous locomotor activity. Spontaneous locomotor activity was automatically measured by a laboratory animal movement analyzing system (ACTIMO-100; Shinfactory, Fukuoka, Japan). Locomotor activity was measured as ambulatory counts from a record of consecutive adjacent infrared beam breaks. Mice were housed individually in plastic cages, and food and water were available *ad libitum*. Mice were acclimatized to the cages for 1 h before recordings commenced and then monitored for 21 h (dark period for 11 h; 01:00 p.m. to 12:00 a.m. and light period for 10 h; 12:00 a.m. to 10:00 a.m.).

In the above behavior tests, the different treatment groups were tested in counterbalanced order with a single blinded method.

RNA extraction. Total RNA was extracted from the whole brain by using QIAzol Lysis Reagent (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Isolated RNA was purified using the RNeasy® Lipid Tissue Mini kit (Qiagen).

DNA microarray analysis. Pooled RNAs were subjected to cRNA synthesis for a DNA microarray analysis. Cyanine-3 (Cy3) labeled cRNA was prepared from 100 ng RNA using the One-Color Low Input Quick Amp labeling kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. All procedures of hybridization, slide washing, and scanning were carried out according to the manufacturer's instructions [Agilent Whole Mouse Genome Microarray kit ver2.0 (G4846A); Agilent Technologies]. The data were analyzed using GeneSpring software version 12.6.1 (Agilent Technologies).

Real-time PCR. cDNA was synthesized from total RNA using the Revertrace RT-PCR kit (Toyobo Co., Ltd., Osaka, Japan). Real-time PCR was performed on an Opticon 2 system (Bio-Rad) using SYBR qPCR mix (Toyobo Co., Ltd.) employing primers (forward/reverse) as shown in Table I. Expression of the target genes was normalized to that of *GAPDH* as an endogenous control gene.

Statistical analysis. Data were analyzed by one-way ANOVA or two-way repeated-measures ANOVA followed by Tukey-Kramer honest significant difference (HSD) test.

Table I. Primer sequences used for real-time PCR.

Gene	Forward (5'-3')	Reverse (5'-3')
S100a8	ACAAGGAAATCACCATGCCCT	TCACCATCGCAAGGAACTCC
S100a9	ACCAGGACAATCAGCTGAGC	ACAGCCTTTGCCATGACTGT
Gpr35	TCTTCCCCCTGGAGATCTTT	CTGGGAGAAAGGAGACCACA
Cyp2e1	TCCCTAAGTATCCTCCGTGA	GTAATCGAAGCGTTTGTTGA
Adh1	TGTGGTTGATGCAACGGTTG	TTCGCGCATAAAAATGCCCC
Adh2	AGGCCAATCTTGCCAGAGTC	GCCAAAGACAGCACAAGTGG
Adh3	CTGGACGAATCCTCCTCCGTAGC	GACTGACAGGCCAACTCCTC
Adh4	AGGCCAATCTTGCCAGAGTC	GCCAAAGACAGCACAAGTCC
Aldh1	GCACTCAATGGTGGGAAAGT	TTTGGCCACACACTCCAATA
Aldh2	GCTGGGCTGACAAGTACCAT	TTGATCAAGTTGGCCACGTA

Table II. Effects of ethanol exposure on SAMP8 mice.

Variable	Control (no ethanol)	1% Ethanol	2% Ethanol
Final body weight (g)	30.6±0.8	28.6±0.8	30.4±0.7
Gains in body weight (g)	6.5±0.5	4.6±0.9	6.5±0.7
Epididymal adipose tissue (g)	0.250±0.040	0.156±0.028	0.190±0.028
Perinephric adipose tissue (g)	0.088±0.016	0.061±0.012	0.080±0.015
Gastrocnemius muscle (g)	0.111±0.006	0.111±0.006	0.117±0.004
Total food intake (g)	451±12	467±7	462±6
Total fluid intake (g)	691±30	680±22	697±31

Values are mean ± SE (n=8).

The level of significance was set at P<0.05. In tables and figures, the means in the row or bar with superscripts without a common letter significantly differ, P<0.05 (Tukey-Kramer HSD test).

Results

Growth and senescence grading score. Food and fluid intake, body weight, and weights of adipose tissues and gastrocnemius muscle weight were not significantly different among the three groups (Table II). Mean ethanol intake in the 2%-ethanol group was almost twice as much as that in the 1%-ethanol group (Table III). Licking counts of drinking water (access status to water bottle) in 20-week-old mice are indicated in Table IV. The temporal changes in the senescence grading score (behavior, skin and hair, eyes, spondylus, and total) are shown in Table V. In 18-week-old mice, the senescence score of behavior and total senescence score were unaffected by ethanol intake. In contrast, in 22-week-old mice, both 1% and 2%-ethanol intake significantly (P<0.05) decreased the senescence scores for behavior and total scores compared to the controls. In 25-week-old mice, 1%-ethanol intake caused lower scores of behavior and total scores than the other two groups (P<0.05). The senescence scores of skin and hair were significantly lower (P<0.05) in the 2%-ethanol group than in the control groups in 18-week-old mice, but there was no difference between the control and 1%-ethanol groups. Ethanol intake caused no influence on the senescence score of spondylus in 25-week-old mice.

Behavioral analyzes. In the open-field test, the rearing activity of animals in the 1%-ethanol group was significantly higher (+77%, P<0.05) than for the control and 2%-ethanol groups. There was no difference in activity between control and 2%-ethanol groups (Fig. 1), indicating that exploratory activity (index of seeking behavior) was increased in the 1%-ethanol group. Moreover, 2%-ethanol intake significantly elevated (+75%, P<0.05, Fig. 1) spontaneous locomotor activity, whereas 1%-ethanol intake did not increase such activity, implying the vitality of 2% ethanol-treated mice. The animals allowed free access to food and drinking, mainly from 01:00 p.m. to 08:00 p.m. in the dark period in this study, which was confirmed by drink sensor measurements (Table IV). The open-field test was conducted from 01:00 p.m. to 03:00 p.m., and it is unclear that the effects of ethanol exposure on the behavioral parameters are direct or indirect effects.

Serum parameters. None of the three groups exhibited significant differences in serum triglyceride, total cholesterol, or glucose levels, and AST and ALT activities were similar among the three groups (Table VI). The serum levels of albumin were significantly lower (-8%, P<0.05) in

Table III. Mean of ethanol ingestion.

Age of mice	Ethanol ingestion (g/kg body weight^{-1} day^{-1})		
	Control (no ethanol)	1% Ethanol	2% Ethanol
10-week-old	0[a]	1.72±0.07[b]	3.34±0.12[c]
11-week-old	0[a]	1.65±0.07[b]	3.17±0.13[c]
13-week-old	0[a]	1.62±0.05[b]	3.10±0.24[c]
15-week-old	0[a]	1.47±0.04[b]	2.93±0.13[c]
17-week-old	0[a]	1.47±0.04[b]	2.88±0.20[c]
19-week-old	0[a]	1.45±0.07[b]	3.17±0.43[c]
21-week-old	0[a]	1.47±0.06[b]	2.98±0.19[c]
23-week-old	0[a]	1.42±0.06[b]	2.69±0.15[c]
25-week-old	0[a]	1.41±0.06[b]	2.83±0.19[c]
26-week-old	0[a]	1.44±0.06[b]	2.89±0.14[c]

Values are mean ± SE (n=8). [a-c]Significantly different by Tukey-Kramer honest significant difference test (P<0.05).

Table IV. Mean of licking counts of drinking water from 11:00 a.m. to 08:00 a.m.

Light/dark period, time range	Licking counts (counts/h)		
	Control (no ethanol)	1% Ethanol	2% Ethanol
Light period, 11:00 a.m. to 12:00 p.m.	7.1±3.4	12.4±1.2	7.9±0.2
Dark period, 12:00 a.m. to 01:00 p.m.	8.1±2.9	7.4±5.1	5.4±2.6
Dark period, 01:00 p.m. to 02:00 p.m.	26.4±8.1	20.6±2.9	13.9±4.0
Dark period, 02:00 p.m. to 03:00 p.m.	19.6±5.0	25.9±4.9	11.9±4.5
Dark period, 03:00 p.m. to 04:00 p.m.	16.8±5.4	19.6±6.4	12.7±6.0
Dark period, 04:00 p.m. to 05:00 p.m.	23.1±7.1	21.6±5.6	12.1±3.7
Dark period, 05:00 p.m. to 06:00 p.m.	17.8±5.4	13.6±4.9	21.0±4.9
Dark period, 06:00 p.m. to 07:00 p.m.	12.8±4.2	22.9±5.7	17.7±5.0
Dark period, 07:00 p.m. to 08:00 p.m.	12.1±4.7	18.8±7.2	10.4±9.7
Dark period, 08:00 p.m. to 09:00 p.m.	9.0±4.2	13.5±7.4	12.1±7.2
Dark period, 09:00 p.m. to 10:00 p.m.	4.5±3.0	7.5±3.5	10.4±6.6
Dark period, 10:00 p.m. to 11:00 p.m.	4.8±3.1	7.0±3.3	5.7±4.6
Dark period, 11:00 p.m. to 12:00 a.m.	10.8±3.8	1.4±3.3	11.3±5.9
Light period, 00:00 a.m. to 01:00 a.m.	1.6±1.5	6.8±0.8	5.4±2.7
Light period, 01:00 a.m. to 02:00 a.m.	0.0±0.0	0.6±2.7	4.3±6.8
Light period, 02:00 a.m. to 03:00 a.m.	0.0±0.0	0.4±0.5	2.9±3.7
Light period, 03:00 a.m. to 04:00 a.m.	1.8±1.6	2.5±0.4	4.1±2.8
Light period, 04:00 a.m. to 05:00 a.m.	2.8±2.2	0.1±2.4	1.7±2.9
Light period, 05:00 a.m. to 06:00 a.m.	3.1±3.1	0.6±0.1	2.4±2.2
Light period, 06:00 a.m. to 07:00 a.m.	0.3±0.2	0.0±0.5	0.0±1.7
Light period, 07:00 a.m. to 08:00 a.m.	2.4±2.4	0.3±0.0	0.0±2.4

Values are mean ± SE (n=8, 20-week-old).

the ethanol group than in the control group, but there was no difference between the control and 2%-ethanol groups (Table VI). Intake of 1% ethanol slightly decreased serum level of insulin (-12%, P<0.01), but that of 2% ethanol did not (Table VI). Serum levels of adiponectin, IGF-1, IL-1β, IL-12, and TNF-α were unaffected by ethanol intake (Table VI).

Table V. Effects of ethanol exposure on senescence grading score in SAMP8 mice.

Variable	Week-old	Control (no ethanol)	1% Ethanol	2% Ethanol	Two-way repeated-measures (ANOVA; P-value)		
					Week-old effect	Ethanol effect	Interaction
Behavior	18	0.03±0.03	0.03±0.03	0			
	22	0.51±0.14[a]	0.08±0.04[b]	0.13±0.07[b]	<0.01	<0.01	<0.01
	25	0.94±0.15[a]	0.30±0.16[b]	0.83±0.10[a]			
Skin and hair	18	0.10±0.04[a]	0.03±0.02[a,b]	0[b]			
	22	0.28±0.04	0.24±0.03	0.30±0.06	<0.01	0.17	0.40
	25	0.71±0.11	0.56±0.04	0.70±0.03			
Eyes	18	0	0	0			
	22	0.15±0.12	0	0	0.24	0.25	0.23
	25	0	0	0			
Spondylus	18	0.13±0.04	0.10±0.03	0.04±0.03			
	22	0.19±0.03	0.16±0.04	0.15±0.03	<0.01	0.16	<0.05
	25	0.54±0.08	0.34±0.05	0.48±0.03			
Total	18	0.25±0.08	0.15±0.06	0.04±0.03			
	22	1.13±0.18[a]	0.48±0.09[b]	0.58±0.10[b]	<0.01	<0.01	<0.01
	25	2.19±0.31[a]	1.20±0.20[b]	2.00±0.10[a]			

Values are mean ± SE. [a,b]Significantly different by Tukey-Kramer honest significant difference test (P<0.05).

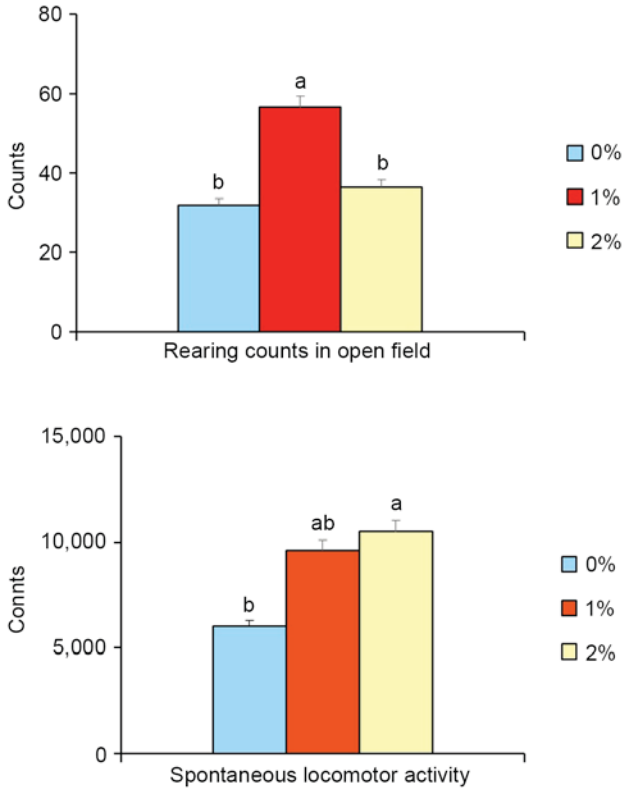

Figure 1. Effects of ethanol on open-field test and spontaneous locomotor activity in SAMP8 mice. Rearing counts in open-field tests at 23-week-old are shown (above). Spontaneous locomotor activity for 17-week-old animals is shown (below). Values are mean ± SE (n=8). One-way ANOVA analysis indicated significant effects of ethanol exposure on the rearing counts and spontaneous locomotor activity (P<0.05). [a,b]Significantly different by Tukey-Kramer honest significant difference test (P<0.05).

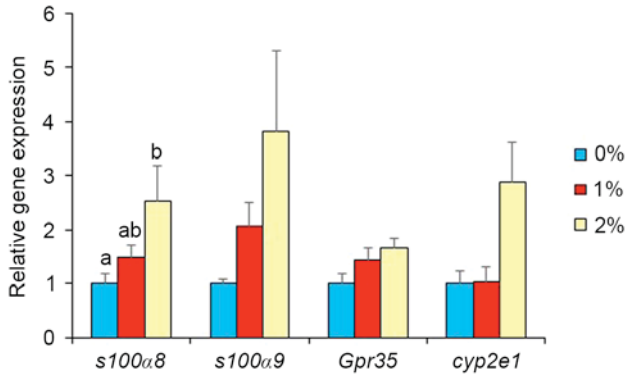

Figure 2. Effects of ethanol exposure on expression of genes related to inflammation and oxidative stress in the brains of SAMP8 mice. Values are mean ± SE (n=5-7). One-way ANOVA analysis indicated significant effects of ethanol exposure on *S100a* expression (P<0.05), but no such effects on expression of other genes. [a,b]Significantly different by Tukey-Kramer honest significant difference test (P<0.05).

Gene expression in brain. In our preliminary study, DNA microarray analysis indicated alterations in the gene expression of *S100a8, S100a9, GPR35, Cyp2e1, Adh1,* and *Adh4* by ethanol intake. Thus, real-time PCR analysis was used in the present study to confirm these results. Gene expression of other ethanol-metabolizing enzymes was also determined. Intake of 2% ethanol resulted in a 2.5-fold elevation (P<0.05; Fig. 2) of *S100a8* mRNA, but 1%-ethanol intake did not. *S100a9, GPR35* and *Cyp2e1* expression levels were unaffected in the 2%-ethanol intake group. Intake of 1% ethanol caused a marked elevation (10-fold, P<0.05; Fig. 3)

Table VI. Effects of ethanol exposure on serum parameters in SAMP8 mice.

Variable	Control (no ethanol)	1% Ethanol	2% Ethanol
Glucose (mmol/l)	8.97±0.53	8.19±0.59	8.59±0.65
Triglyceride (mmol/l)	1.18±0.13	0.99±0.05	1.10±0.09
Total cholesterol (mmol/l)	2.72±0.12	2.65±0.10	2.74±0.15
ALT (U/l)	25.3±1.5	26.5±2.1	26.3±1.6
AST (U/l)	133±5	124±9	135±6
Albumin (g/l)	27.0±0.4[a]	24.8±0.6[b]	25.5±0.7[a,b]
Insulin (mg/l)	0.63±0.02[a]	0.55±0.01[b]	0.58±0.01[a,b]
Adiponectin (mg/l)	6.51±0.22	7.11±0.41	7.63±0.22
IGF-1 (μg/l)	310±39	272±35	237±38
IL-1β (ng/l)	407±94	376±94	308±87
IL-12 (ng/l)	128±33	97±33	80±31
TNF-α (ng/l)	312±68	272±68	211±63

Values are mean ± SE (n=6-8). [a,b]Significantly different by Tukey-Kramer honest significant difference test (P<0.05). ALT, alanine aminotransferase; AST, aspartate aminotransferase; IGF, insulin-like growth factor; IL, interleukin; TNF, tumor necrosis factor.

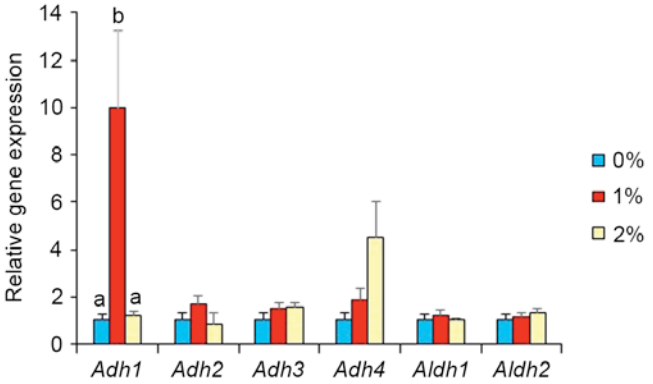

Figure 3. Effects of ethanol exposure on expression of genes involved in alcohol metabolism in the brain of SAMP8 mice. Values are mean ± SE (n=5-7). One-way ANOVA analysis indicated significant effects of ethanol consumption on *Adh1* expression (P<0.05), but no similar effects on expression of other genes. [a,b]Significantly different by Tukey-Kramer honest significant difference test (P<0.05).

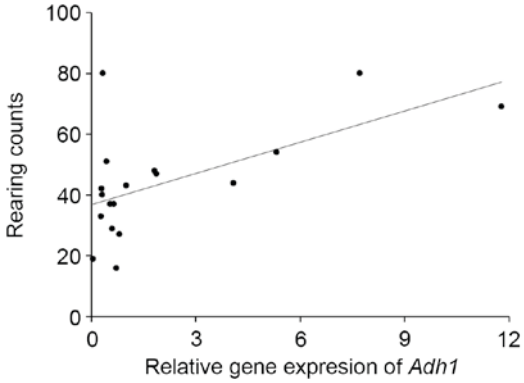

Figure 4. Correlation of relative gene expression of *Adh1* and rearing activity in SAMP8 mice.

in *Adh1* expression, but that of 2% ethanol did not. Intake of 1 and 2% ethanol caused no influence on *Adh2, Adh3, Adh4, Aldh1, and Aldh2* expression (Fig. 3). *Adh1* expression was significantly correlated with the rearing activity of the mice (r=0.598, P<0.01; Fig. 4) and with the total senescence score at 22 weeks (r=-0.497, P<0.05), but not with the total senescence score at 25 weeks (r=-0.412, P=0.09). The expression of *Adh1* was not correlated (P>0.05) with any of the serum factors or behavioral results, with the exception of rearing activity. In addition, the serum results were not correlated with the rearing activity and total senescence scores (P>0.05). *Adh2, Adh3, Aldh1* and *Aldh2* expression levels were unaffected by ethanol intake. DNA microarray analysis also indicated the elevated gene expression of several olfactory receptors as a consequence of 1% ethanol intake (Fig. 5).

Discussion

The present results, obtained SAMP8 mice, indicate that low-ethanol intake does not exert any significant deleterious effects on the general welfare of animals. Consumption of 1% ethanol appeared to retard senescence development with respect to the eyes, skin, and hair, and behavior, whereas 2%-ethanol intake appeared to do so to a lesser extent. These results suggest that 1%-ethanol intake is beneficial for SAMP8 mice.

Here, indices of liver function in SAMP8 mice were unaffected by ethanol intake. This is in contrast to the results observed in the rats fed a high-fat diet, in which 1%-ethanol intake improved the parameters relating to the liver function (10). Although the reason for this discrepancy is unknown, our study implies a favorable effect of 1%-ethanol intake on SAMP8 animals, which may be mediated through mechanisms not involving liver function. Of interest is the finding that 1%-ethanol intake caused a significant reduction in serum insulin, which has been considered to play an important role in aging

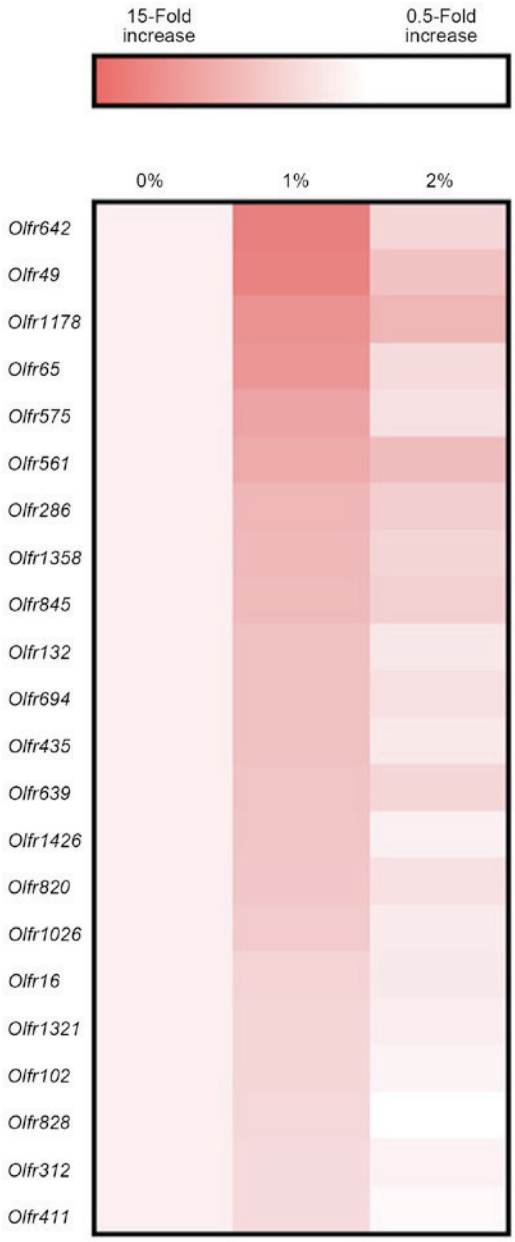

Figure 5. Effects of ethanol exposure on expression levels of several olfactory receptor genes in the brain of SAMP8 mice. Pooled samples from three groups were employed for the analysis using DNA microarray.

process (18), whereas 2% ethanol did not. However, serum insulin levels were not associated with the total senescence score, raising activity and *Ahd1* expression. Further study is necessary to examine the effect of 1% ethanol on insulin signaling in the senescence mice.

In this study, analysis using open-field tests demonstrated a significant elevation in rearing activity in the 1%-ethanol group, but not in the 2%-ethanol group. This rearing activity has been suggested an index of exploratory behavior (19,20). Importantly, senescence has previously been reported to be associated with diminished rearing activity (21,22). Thus, at low doses of ethanol, ethanol is likely to cause positive effects on such 'seeking-out' behavior, which is otherwise decreased by senescence. Because senescence is associated with decreased locomotor function in SAMP1 animals (23), locomotor function was also examined. We found that 2%-ethanol intake significantly elevated (+75%) locomotor activity, whereas 1%-ethanol intake tended to promote such activity to a lesser degree (+60%). The results were consistent with the previous studies indicating low doses of ethanol stimulate locomotor activity in mice (24). Thus, intake of either 1 or 2% ethanol appears to have positive effect on the locomotor function in SAMP8 mice.

Gene expression analysis revealed significantly higher levels of brain *S100a8* in the 2%-ethanol group, but not in the 1%-ethanol group. *S100a9, GPR35* and *Cyp2e1* expressions also tended to be higher in the 2%-ethanol group. *S100a8* and *S100a9* have been suggested to be involved in inflammatory signaling (25), and GPR35 is proposed to be associated with inflammation (26). *Cyp2e1* is considered a source of reactive oxygen species generation (27). Thus, the dose of 2% ethanol appears to be necessary for the induction of expression of the factors responsible for inflammation and oxidative stress.

Surprisingly, our study quantified a marked elevation in gene expression in brain tissue for *Adh1* in the 1%-ethanol group, but not in the 2%-ethanol group. Alcohol dehydrogenases (ADHs) metabolize a broad spectrum of substrates such as alcohols and aldehydes endogenously produced during lipid peroxidation so as to prevent the possible toxic accumulation of these compounds (28). Because these compounds can be harmful to dopaminergic neurons, ADHs have attracted attention. Genetic variants in ADH1C have been reported to be associated with Parkinson disease (29). In fact, recent study using *Adh1* knockout mice has shown lack of *Adh1* leads to changes in dopamine neurons related behavior (30). Furthermore, *Adhs* are a critical mediator of retinoic acid synthesis from vitamin A (31,32). Retinoic acid has been suggested a protective factor against neurodegenaration via retinoid signaling (33). Our studies further indicated *Adh1* expression is significantly correlated with the rearing activity. Expression of several olfactory receptor genes was also higher in the 1%-ethanol group compared with other groups. An Alzheimer's disease model rat revealed down regulation of olfactory receptor genes in the olfactory bulb (34) and olfactory dysfunction has been also reported in neurodegerative disorders such as Alzheimer's and Parkinson's diseases. Olfactory dysfunction also increases with aging. In view of these facts, it will be necessary to evaluate if perturbed expression of *Adh1* expression leads to the alterations in the rearing activity. Furthermore, the elevation of *Adh1* expression requires confirmation at the protein level and is being investigated in future studies.

We obtained preliminary measurement data for serum ethanol when dissected (01:00 p.m. to 03:00 p.m.) at 23-week-old, noting that no differences were observed among the three groups (Kimoto *et al*, unpublished data). At present, there are no supporting data from the literature to suggest what blood or brain ethanol concentrations may have been reached in this model as a result of the 1 and 2% ethanol treatments. It has been reported that consumption of 6% ethanol containing liquid diet by C56BL6 mice for 22 weeks permits the use of plasma ethanol as a confirmation of alcohol exposure model (35). Meanwhile, plasma ethanol levels of the mice fed 3% ethanol containing liquid diet did not significantly differ from the base line levels of mice without receiving ethanol (35).

In conclusion, our study provides evidence for the beneficial effect of low doses of ethanol on SAMP8 mice. In particular, 1%-ethanol intake appeared to cause a favorable effect on

senescence score and rearing activity, whereas 2%-ethanol intake prompted a lesser effect. These results support the J-curve effect for ethanol exposure as suggested by a number of epidemiological studies (1-3). Of great interest is the finding of the markedly higher *Adh1* expression in the brains of 1%-ethanol group, but not in those of the 2%-ethanol exposed group. Thus, our results raised the prospect that the induction of *Adh1* expression by 1% ethanol intake leads to the quantified beneficial effect. Further research is necessary to examine this proposal. The molecular mechanisms modulating higher levels of *Adh1* expression by 1% ethanol also warrant further investigation. At present, it is unclear whether the 1% ethanol intake exerted direct or indirect effect on the *Adh1* expression and the rearing activity. Further study will be necessary to reveal this issue.

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