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 neurodegeneration. 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.

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-prefering 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 diethylether anesthesia (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 Revertrue 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 1. 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.
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 I. Primer sequences used for real-time PCR.

| Gene      | Forward (5’-3’)          | Reverse (5’-3’)          |
|-----------|--------------------------|--------------------------|
| S100a8    | ACAAGGAAATCACCATGCCCCT   | TCACCAGCGAAGGACTCC       |
| S100a9    | ACCAGGACAATCGCTGAGC      | ACGCCTTTGCCCAGTACTGT     |
| Gpr35     | TCTCCCCCTTGGAGATCTTT     | CTGGGGAAGAAGGAGACCACA    |
| Cyp2e1    | TCCCTAAATCTCTCCTCGTGA   | GAATCGAGCTTTGGTGA        |
| Adh1      | TGGGTGTTAGCACAAGGTG      | TCGGCCATAAAATGCCC       |
| Adh2      | AGCCCAATCTTGGCCAGAGTC    | GCCAAGACAGCAGCAGTGG      |
| Adh3      | CTGGGACAGCACTCCCTCCGTC   | GACGTGACAGCGAAGCTCCTC   |
| Adh4      | AGCCCAATCTTGGCCAGAGTC    | GCCAAGACAGCAGCAGTGG      |
| Aldh1     | GCACCTCAATGTTGGGAAAGT    | TTTGCCCACACTCCAAATA      |
| Aldh2     | GCTGGGCTGCAAGTACCAT      | TTGATGAGGTGGCACAGTA      |

### 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)           | 45±1±2               | 46±7±2     | 46±6±2     |
| Total fluid intake (g)          | 691±30               | 680±22     | 697±31     |

Values are mean ± SE (n=8).
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).
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 V. Effects of ethanol exposure on senescence grading score in SAMP8 mice.

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

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).
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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).

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±0.4a            | 24.8±0.6b  | 25.5±0.7ab |
| Insulin (mg/l) | 0.63±0.02a           | 0.55±0.01b | 0.58±0.01ab|
| 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.

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
and -

23
30
34
ined. We found that 2%-ethanol intake significantly elevated
in SAMP1 animals (43). Senescence is associated with decreased locomotor function, which is otherwise decreased by senescence. Because ethanol is likely to cause positive effects on such 'seeking-out'
diminished rearing activity (44). Locomotor function was also exam-
ined. 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 peroxida-
tion 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 ADHIC have been reported to be associ-
ated 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 neurodegeneration via retinoid signaling (33).
Our studies further indicated Adh1 expression is significantly
correlated with the rearing activity. Expression of several olfac-
tory 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 altera-
tions 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

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

1. Marmot MG, Rose G, Shipley MJ and Thomas BF: Alcohol and mortality: A U-shaped curve. Lancet 1: 580-583, 1981.
2. Di Castelnuovo A, Costanzo S, Bagnardi V, Donati MB, Iacoviello L and de Gaetano G: Alcohol dosing and total mortality in men and women: An updated meta-analysis of 34 prospective studies. Arch Intern Med 166: 2437-2445, 2006.
3. Nova E, Baccan GC, Vesas A, Zapatera B and Marcos A: Potential health benefits of moderate alcohol consumption: Current perspectives in research. Proc Nutr Soc 71: 307-315, 2012.
4. Mukamal KJ, Killmer LH, Fitzpatrick AL, Longstreth WT Jr, Mittleman MA and Siscovick DS: Prospective study of alcohol consumption and risk of dementia in older adults. JAMA 289: 1405-1413, 2003.
5. Peters R, Peters J, Warner J, Beckett N and Bulpitt C: Alcohol, dementia and cognitive decline in the elderly: A systematic review. Age Ageing 37: 505-512, 2008.
6. Fagrell B, De Faire U, Bondy S, Crichton M, Gaziano M, Gronbaek M, Jackson R, Klatsky A, Salonen J and Shaper AG: The effects of light to moderate drinking on cardiovascular diseases. J Intern Med 246: 331-340, 1999.
7. McCann SE, Sempos C, Friedland EH, Brindis CD, McLean R, Kreiger N, Freeman MP, Novotny TE, Subramaniam M and Ziegler GR: Mortality in men and women: An updated meta-analysis of 34 prospective studies. Arch Intern Med 166: 2437-2445, 2006.
8. Izu H, Shobayashi M, Manabe Y, Goto K and Iefuji H: Age-related alterations in the metabolic profile in the hippocampus of the senescence-accelerated mouse prone 8: A spontaneous Alzheimer’s disease mouse model. J Alzheimers Dis 39: 841-848, 2014.
9. Shimozaki M, Takahashi Y, Mukaino M, Saito N, Toyama Y, Okumura H and Nakamura M: Novel concept of motor functional analysis for spinal cord injury in adult mice. J Biomed Biotechnol 2011: 157458, 2011.
10. Lamming DW: Diminished mTOR signaling: A common mode of action for endocrine longevity factors. Springerplus 3: 735, 2014.
11. Easton A, Arbusova J and Turek FW: The circadian Clock mutation increases exploratory activity and escape-seek behavior. Genes Brain Behav 2: 11-19, 2003.
12. Pardo M, Lopez-Cruz L, Valverde O, Ledent C, Baqui Y, Muller CE, Salamone JD and Correa M: Effect of subtype-selective adenosine receptor antagonists on basal or haloperidol-regulated striatal function: Social effects of exploratory locomotion and c-Fos immunoreactivity in outbred A(2A)R KO mice. Behav Brain Res 247: 226-227, 2013.
13. Ikeyama S, Shumiya S and Kawamura H: Age-related changes in radial-arm maze learning and basal forebrain cholinergic systems in senescence accelerated mice (SAM). Behav Brain Res 51: 15-22, 1992.
14. Lalonde R and Strazielle C: Exploratory activity and motor coordination in old versus middle-aged C57BL/6 J mice. Arch Gerontol Geriatr 49: 39-42, 2009.
15. Arizzi MN, Correa M, Betz AJ, Wisniewski A and Salamone JD: Behavioral effects of intraventricular injections of low doses of ethanol, acetaldheyde and acetate in rats: Studies with low and high rate operant schedules. Behav Brain Res 147: 203-210, 2003.
16. Gebhardt C, Németh J, Angel P and Hess J: S100A8 and S100A9 in inflammation and cancer. Biochem Pharmacol 72: 1622-1631, 2006.
17. Divvy N, MacKenzie AE, Nicklin SA and Milligan G: G protein-coupled receptor 35: An emerging target in inflammatory and cardiovascular disease. Front Pharmacol 6: 41, 2015.
18. Seitz HK and Wang XD: The role of cytochrome P450 2E1 in ethanol-mediated carcinogenesis. Subcell Biochem 67: 113-134, 2013.
19. Boleda MD, Saubin N, Farrés J and Parés X: Physiological substrates for rat alcohol dehydrogenase classes: Aldehydes of lipid peroxidation, omega-hydroxyfatty acids and retinoids. Arch Biochem Biophys 307: 85-90, 1993.
20. Buervenich S, Sylow O, Carmine A, Zhang Z, Anvret M and Olson L: Alcohol dehydrogenase alleles in Parkinson's disease. Mov Disord 15: 813-818, 2000.
21. Anvret A, Ran C, Westerlund M, Gellhaar S, Lindqvist E, Pernold K, Lundströmer K, Duester G, Felder MR, Galter D and Belin AC: Adh1 and Adh1/4 knockout mice as possible rodent models for presymptomatic parkinson's disease. Behav Brain Res 247: 217-226, 2013.
22. Duester G: Alcohol dehydrogenase as a critical mediator of retinoid acid synthesis from vitamin A in the mouse embryo. J Nutr 128 (2 Suppl): 459S-462S, 2008.
23. Molotkov A, Deltour L, Foglio MH, Cuenca AE and Duester G: Distinct retinoid metabolic functions for alcohol dehydrogenase genes Adh1 and Adh4 in protection against vitamin A toxicity or deficiency revealed in double null mutant mice. J Biol Chem 277: 13804-13811, 2002.
24. Sokolof RR and Singh N: Retinoids as potential targets for Alzheimer’s disease. Pharmacol Biochem Behav 120: 117-123, 2014.
25. Zhu YY, Ni DF and Xu CM: Gene expression profiles in the olfactory bulb from a rat model of Alzheimer’s disease. J Alzheimers Dis 18: 581-593, 2009.
26. Emmons EE, Manaves V, Singer T and Tabesh T: Chronic alcohol feeding inhibits atherogenesis in C57BL/6 hyperlipidemic mice. Am J Pathol 147:1749-1758, 1995.