S-Adenosylmethionine (SAM)-Accumulating Sake Yeast Suppresses Acute Alcohol-Induced Liver Injury in Mice

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The suppressive effects on acute alcoholic liver injury of S-adenosylmethionine (SAM) and the sake yeast, Saccharomyces cerevisiae Kyokai No. 9, have been shown previously. To enhance the suppression of acute alcoholic liver injury by sake yeast, we prepared SAM-accumulating sake yeast (SAM yeast). Male C57BL/6 mice that had been fed on a diet containing 0.25% SAM yeast or sake yeast for two weeks received three doses of ethanol (5 g/kg BW). In the mice fed on the SAM yeast, the ethanol-induced increases in both triglyceride (TG) and alanine aminotransferase (ALT) were significantly repressed. In addition, the SAM yeast-fed mice did not show an ethanol-induced decrease in hepatic SAM level, suggesting that a disorder of methionine metabolism in the liver caused by ethanol was relieved by the SAM yeast. These results suggest that the SAM yeast had a stronger effect suppressing acute alcoholic liver injury in mice than the sake yeast.

Key words: Saccharomyces cerevisiae; sake yeast; liver injury; alcohol; S-adenosylmethionine

S-Adenosylmethionine (SAM) is an important molecule in normal cell functions;1) it is (i) a regulatory compound of methionine metabolism; (ii) a precursor of glutathione synthesis through the transsulfuration pathway; (iii) a precursor of polyamine synthesis; and (iv) a major methyl group donor in the transmethylation of proteins, nucleic acids, polysaccharides, phospholipids and fatty acids. Abnormalities in SAM metabolism with liver disease are well recognized.1) SAM administration reduces liver damage caused by ethanol,2) d-galactosamine,3) and acetaminophen4) in mice and rats, and improves alcoholic liver disease in humans.5,6) SAM has the potential to treat alcoholic liver injury by acting as a precursor of the antioxidant, glutathione, repairing the mitochondrial glutathione level, attenuating the effects of proinflammatory cytokines, and increasing DNA methylation.5,6) Besides liver injury, SAM has also been suggested by clinical research to be a chemotherapeutic agent in various diseases such as depression,7) osteoarthritis,8) and Alzheimer’s disease.9)

Sake yeast, Saccharomyces cerevisiae Kyokai yeast, which is used for sake (Japanese rice wine) brewing shows an unusual intracellular accumulation of SAM. Compared with other yeasts, bacteria, molds, and some other microorganisms, the content of accumulated SAM is higher in sake yeast.10,11) The experimental culture conditions for SAM production by sake yeast have been optimized and it was revealed that the addition of l-methionine was required.10,11) SAM may offer considerable advantages in comparison to sake yeast as a nutritional resource due to its biological significance. Interestingly, an intracellular accumulation of SAM in sake yeast has also been observed during sake brewing,12) indicating that the conditions that sake brewing are suitable for SAM accumulation in sake yeast. Sake lees, a brewing by-product of sake, contain SAM derived from sake yeast, because sake lees contain rice components that are not assimilated by sake yeast and sake koji, Aspergillus oryzae, the components of yeast and sake koji, and their metabolites. The demand for sake lees is limited and some are discarded as industrial waste. Therefore, understanding the physiological effects of sake yeast may help in understanding the physiological effects of sake lees and finding new uses for them.

We have shown that sake yeast suppressed acute alcoholic liver injury in mice.13) In mice fed sake yeast, ethanol-induced increases in triglyceride (TG) and alanine aminotransferase (ALT) were significantly attenuated and hepatic steatosis was improved.13) Ethanol consumption causes abnormal methionine metabolism, because ethanol consumption inhibits the activity of methionine synthase1) which is responsible for the conversion of homocysteine (Hcy) to methionine. SAM is converted from methionine catalyzed by methionine adenosyltransferase. The inhibition of methionine synthesis by ethanol leads an increase in the hepatic Hcy level and a decrease in the hepatic methionine level.

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Abbreviations: TG, triglyceride; ALT, alanine aminotransferase; SAM, S-adenosylmethionine; Hcy, homocysteine; SGF, simulated gastric fluid; BW, body weight
which results in a decrease in the hepatic SAM level.\textsuperscript{14–18} \textit{Sake} yeast-fed mice have shown a smaller decrease in hepatic SAM level and a smaller increase in plasma Hcy level after an ethanol treatment than control mice,\textsuperscript{13} suggesting that sake yeast protected the liver from abnormal methionine metabolism due to ethanol administration. We expected that SAM accumulated in sake yeast would enhance the suppressive effect of sake yeast on acute alcoholic liver injury, because SAM suppresses liver damage. We examine here the effect of SAM-accumulating sake yeast (SAM yeast) on acute alcoholic liver injury, and compare the effect with that of sake yeast.

\textbf{Materials and Methods}

\textit{Yeast strains and culture conditions.} The yeast strains used in this study were \textit{Saccharomyces cerevisiae} K-9 (Kyokai No. 9) as a sake yeast and \textit{Saccharomyces cerevisiae} X2180-1A as a laboratory yeast. A YPD medium (2\% yeast extract, 1\% peptone, and 2\% glucose) was used for yeast cultivation. \textit{S. cerevisiae} K-9 and X2180-1A cells were cultivated aerobically at 30°C. The cells were collected and washed three times with sterilized distilled water. These cells were kept at −20°C and lyophilized (−20°C, 16 hr) to prepare the animal diets.

\textit{Preparation of SAM yeast.} The yeast strain used for the accumulation of SAM was \textit{Saccharomyces cerevisiae} K-9. The culture conditions developed by Shiozaki\textsuperscript{10,11} with some modifications, were utilized to accumulate SAM in K-9 cells. In brief, 750 ml of a D medium (5\% sucrose, 0.3\% yeast extract, 0.4\% KH\textsubscript{2}PO\textsubscript{4}, 0.2\% K\textsubscript{2}HPO\textsubscript{4}, 0.05\% MgSO\textsubscript{4}·7H\textsubscript{2}O, and 0.75\% l-methionine at pH 6.0) was used for yeast cultivation after adding 30 ml of ethanol. The cells were aerobically cultivated at 30°C, collected and washed three times with sterilized distilled water. The cells were kept at −20°C and lyophilized (−20°C, 16 hr) to prepare the animal diets.

\textit{Animal experiments.} Nine-week-old male C57BL/6 mice (Charles River Japan, Yokohama, Japan) were maintained under controlled conditions (ambient temperature, 22 ± 2°C; relative humidity, 60\%; light condition, 12-h-light/dark cycle, lights on: 0:00 to 12:00). The animals had free access to food and water. All animals received humane care as outlined in the Guide for the Care and Use of Laboratory Animals (National Research Institute of Brewing, Animal Care Committee). After adaptation to a control diet (Table 1) for 7 days, the mice were fed the control diet or an experimental diet containing SAM yeast or sake yeast for 14 days. SAM yeast and sake yeast were prepared as already described and added to the control diet at the expense of an equal amount of casein (Table 1).

\textit{Induction of acute alcoholic liver injury.} The binge drinking mouse model\textsuperscript{2} was utilized for the induction of acute alcohol liver injury. After adaptation, the mice were assigned to six groups: control, ethanol, SAM yeast, SAM yeast/ethanol, sake yeast, and sake yeast/ethanol. For 14 days, the control and ethanol-treated groups were fed on a diet containing 0.25\% SAM yeast, and the sake yeast and sake yeast/ethanol groups were fed on a diet containing 0.25\% sake yeast. The mice in the ethanol, SAM yeast/ethanol and sake yeast/ethanol groups received ethanol (5 g/kg BW) by gavage every 12 hrs for a total of three doses. The mice in the remaining groups received an isocaloric maltose solution instead of ethanol. At 12 hr after the final ethanol dose, the mice were killed by decapitation to obtain their blood and liver. Blood plasma was separated from heparinized whole blood by centrifugation. The plasma and liver obtained were stored at −80°C until needed for analysis.

\textit{Biochemical analysis of the plasma.} The activity of alanine aminotransferase (ALT, EC 1.1.1.27) and the level of triglyceride (TG) were measured colorimetrically by the DRICHEM, commercial assay system (Fuji Film, Tokyo, Japan).

\textit{Determination of SAM in the yeast cells and liver.} The SAM level was quantified by the capillary electrophoresis method as described previously.\textsuperscript{19} Frozen liver was homogenized in 5 volumes (v/wt) of an ice-cold 4\% perchloric acid solution. The homogenate was centrifuged at 13,000 × g for 10 min at 4°C, and the supernatant was used for analysis. Harvested yeast cells were washed three times with distilled water, suspended in a 10\% perchloric acid solution, and centrifuged at 13,000 × g for 10 min at 4°C. The supernatant was subjected to capillary electrophoresis in an AccuSep fused silica 75µm × 60 cm column (Waters, Milford, USA) after appropriate dilution.

\textit{Measurement of water-soluble vitamins.} Water-soluble vitamins (vitamin B1, vitamin B2, vitamin B6, folate, niacin, and pantothenic acid) in the yeast cells were measured by SRL Food Analysis Service (Tokyo, Japan).

\begin{table}[h]
\centering
\caption{Composition of the Experimental Diets (w/w \%)}
\begin{tabular}{llll}
\hline
Ingredient & Control & 0.25\% SAM yeast & 0.25\% Sake yeast \\
\hline
Casein & 25 & 24.75 & 24.75 \\
Cornstarch & 51.5 & 51.5 & 51.5 \\
Sucrose & 5 & 5 & 5 \\
Cellulose & 8 & 8 & 8 \\
Soybean oil & 6 & 6 & 6 \\
Minerals & 3.5 & 3.5 & 3.5 \\
Vitamins & 1 & 1 & 1 \\
Sake yeast & — & 0.25 & — \\
Sake yeast & — & 0.25 & — \\
\hline
\end{tabular}
\end{table}
Japan). Vitamins B1 and B2 were determined by HPLC, and the others by microbial assay systems. The cultivated yeast cells were washed three times with sterilized distilled water and then analyzed.

Incubation of SAM yeast cells in simulated gastric fluid (SGF). To test the digestibility of yeast cells by gastric fluid and the stability of yeast cells in gastric fluid, the cells were incubated in SGF. SAM yeast was cultured in the D medium as already described. After washing with sterilized water, the SAM yeast cells were subjected to digestion as viable SAM yeast cells. After washing with sterilized water and lyophilizing, the SAM yeast cells were subjected to digestion as lyophilized SAM yeast cells. The digestive efficiency of the yeast cells in SGF was estimated according to the content of SAM released from the yeast. The yeast cells were incubated in SGF, which consisted of sterilized water adjusted to pH 1.2 with hydrochloric acid with the digestive enzyme, pepsin (3.2 mg/ml of SGF). The cells were suspended in SGF (2 mg/ml), and incubation was performed at 37°C for 0–120 min. Samples were centrifuged at 13,000 × g for 10 min at 4°C, and SAM was determined in the supernatant after appropriate dilution. As a control, SAM yeast cells were suspended in a 10% perchloric acid solution (2 mg/ml) and then incubated for 1 hr to extract SAM from the cells. The control sample was centrifuged at 13,000 × g for 10 min at 4°C, and SAM was determined in the supernatant after appropriate dilution. The digestive efficiency of the SAM yeast cells at various times was calculated by considering the SAM contents of the perchloric acid solution-treated cells as 100%.

Statistical analysis. Data are expressed as the mean ± SEM. A statistical analysis was performed by one-way ANOVA. Statistical differences were evaluated by the Tukey-Kramer test when the F-value was significant. A p-value of 0.05 or less is considered significant.

Results

Accumulation of SAM in sake yeast

To prepare the SAM yeast, sake yeast was cultured in the D medium containing L-methionine, sucrose, and ethanol as described in the Materials and Methods section. Sake yeast cultured in the D medium respectively contained 173- and 411-fold as much SAM as sake yeast and X2180-1A, a laboratory yeast strain, cultured in the YPD medium (Table 2). We used these culture conditions to prepare SAM yeast for further analysis. An experimental diet containing SAM yeast was made up.

Digestive efficiency of SAM yeast and stability of SAM in lyophilized SAM yeast in SGF

SAM is accumulated in the vacuole of yeast cells, and SAM is released from the yeast after digestion by SGF. At zero time, 81.8% of SAM had already been released from the lyophilized SAM yeast cells (Fig. 1A). However, only 0.23% of the SAM had been released from the viable SAM yeast cells (Fig. 1B). After incubating for 30 min, the digestive efficiency of the lyophilized SAM yeast had reached a plateau (Fig. 1A), but that of viable SAM yeast did not reach a plateau (Fig. 1B). The digestibility of lyophilized the SAM yeast was higher than that of the viable SAM yeast, and lyophilized SAM yeast was more appropriate for supply by feeding.

Table 2. Content of SAM and Water-Soluble Vitamins in SAM Yeast, Sake Yeast, and X2180-1A

<table>
<thead>
<tr>
<th></th>
<th>SAM yeast</th>
<th>Sake yeast</th>
<th>X2180-1A</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM (mg)</td>
<td>78.0</td>
<td>0.45</td>
<td>0.19</td>
</tr>
<tr>
<td>Vitamin B1 (µg)</td>
<td>95.9</td>
<td>371</td>
<td>315</td>
</tr>
<tr>
<td>Vitamin B2 (µg)</td>
<td>50.8</td>
<td>42.2</td>
<td>38.5</td>
</tr>
<tr>
<td>Vitamin B6 (µg)</td>
<td>37.3</td>
<td>28.7</td>
<td>27.9</td>
</tr>
<tr>
<td>Folate (µg)</td>
<td>69.7</td>
<td>23.4</td>
<td>2.62</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>1.25</td>
<td>1.44</td>
<td>0.65</td>
</tr>
<tr>
<td>Pantothenic acid (µg)</td>
<td>163</td>
<td>191</td>
<td>181</td>
</tr>
</tbody>
</table>

(per 1 g dry weight of cells)
Most SAM had already been released from the lyophilized SAM after 5 min of incubation, and the SAM content in SGF did not decrease during further incubation (Fig. 1A). These results indicate that SAM was stable in SGF at 37 °C for 120 min at least.

Stability of SAM in lyophilized SAM yeast during storage
Lyophilized SAM yeast was stored at −20 °C, 4 °C, and 25 °C for 6 months, and SAM was determined by capillary electrophoresis. SAM was stable at −20 °C for 6 months (Fig. 2). SAM was less stable at 4 °C and decreased to 57.0% of its original level after 6 months (Fig. 2). SAM was unstable at 25 °C and decreased to 65.7% of its original level after only 0.5 months (Fig. 2). Finally, SAM decreased to 3.6% of its original level at 25 °C after 6 months (Fig. 2). Thus, it was necessary to store lyophilized SAM yeast at −20 °C to maintain the SAM level.

Table 3. Growth and Food Intake of Mice Fed the Control, SAM Yeast, or Sake Yeast Diet for 14 Days

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.25% SAM yeast</th>
<th>0.25% Sake yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>23.5 ± 0.4</td>
<td>23.1 ± 0.4</td>
<td>23.1 ± 0.5</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>24.5 ± 0.5</td>
<td>25.2 ± 0.5</td>
<td>24.1 ± 0.7</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>2.5 ± 0.1</td>
<td>2.8 ± 2.0</td>
<td>2.8 ± 0.1</td>
</tr>
</tbody>
</table>

Among the control, 0.25% SAM yeast, and 0.25% Sake yeast groups (n = 10), there were no significant differences in growth and food intake.

Suppression of the acute ethanol-induced increases in TG and ALT by SAM yeast and sake yeast
SAM yeast and sake yeast did not affect the growth and food consumption of the mice under the conditions used in this study (Table 3). SAM yeast and sake yeast did not affect the plasma TG and ALT levels in the alcohol-untreated mice (Figs. 3A and B). The ethanol treatment increased the TG and ALT levels by 2.1-fold and 3.8-fold, respectively, as compared with the control group (Figs. 4A and B). These ethanol-induced increases in TG and ALT levels were decreased to 69.4% and 46.3% in the SAM yeast/ethanol group (Figs. 4A and B). The ethanol-induced increases in TG and ALT levels were decreased to 75.7% and 85% in the sake yeast/ethanol group (Figs. 4A and B). These results indicate that both the SAM yeast and sake yeast suppressed the ethanol-induced increases in TG and ALT, but that the suppressive effect of the SAM yeast was greater than that of the sake yeast.

Prevention of the acute ethanol-induced decrease in hepatic SAM level by SAM yeast and sake yeast
SAM yeast and sake yeast did not affect the hepatic
there was no reduced hepatic SAM level in the SAM yeast/ethanol and sake yeast/ethanol groups (Fig. 5). These results suggest that the ethanol-induced decrease in hepatic SAM level was suppressed by the SAM yeast and sake yeast.

Contents of water-soluble vitamins in the SAM yeast, sake yeast and X2180-1A, the laboratory yeast strain

The content of folate was 26.6-fold and 8.9-fold greater, respectively, in the SAM yeast and sake yeast than in X2180-1A (Table 2). The content of niacin was 1.9-fold and 2.2-fold greater in the SAM yeast and sake yeast, respectively, than in X2180-1A (Table 2). The content of vitamin B1 was 3.9-fold and 3.3-fold greater, respectively, in the sake yeast and X2180-1A than in the SAM yeast (Table 2). The contents of vitamin B2, vitamin B6, and pantothenic acid did not differ in these three yeasts (Table 2).

Discussion

SAM is a well-known chemotherapeutic agent for such disorders as alcoholic liver disease, depression, and osteoarthritis, and SAM is a popular nutritional supplement in the United States. The administration of SAM has suppressed the liver injury induced by ethanol, D-galactosamine, and acetoaminophen in mice and rats. Since sake yeast accumulates SAM depending on the culture conditions, sake yeast containing SAM could be expected to efficiently suppress alcoholic liver injury. Therefore, we prepared SAM yeast and examined its suppressive effect on acute alcoholic liver injury.

SAM is an unstable substance and is easily degrade. Before the examination of liver injury, we first determined the stability of SAM in SAM yeast. We confirmed that SAM in lyophilized SAM yeast was stable at $-20^\circ$C for 6 months, but unstable at 25 $^\circ$C (Fig. 2). To prevent any degradation of SAM, lyophilized SAM yeast and a diet containing lyophilized SAM

![Fig. 4](image-url)  
**Fig. 4.** Effects of SAM Yeast and Sake Yeast on the TG and ALT Levels in Alcohol-Treated Mice.  
Mice were fed a control, a SAM yeast-containing, or a sake yeast-containing diet for 14 days. The mice in the EtOH, SAM yeast/ EtOH, and sake yeast/EtOH groups were given 5 g/kg of ethanol three times at 12-hr intervals by gavage, and those in the control group were given a maltose solution instead of alcohol. Blood was collected 12 hrs after the third administration of ethanol. The level of TG (A) and the activity of ALT (B) in the plasma were measured. Each value is expressed as the mean ± SEM (n = 8). Values with different letters are significantly different at p < 0.05 by the Tukey-Kramer test.

![Fig. 5](image-url)  
**Fig. 5.** Effects of SAM Yeast and Sake Yeast on the Hepatic SAM Level in Alcohol-Treated Mice.  
Mice were treated in the same way as described in Fig. 4, the liver was collected 12 hrs after the third administration of ethanol, and the hepatic SAM level was measured by capillary electrophoresis. Each value is expressed as the mean ± SEM (n = 6). Values with different letters are significantly different at p < 0.05 by the Tukey-Kramer test.
yeast were both kept at −20 °C until needed use. Yeast is surrounded by the rigid cell walls, and the digestibility of yeast by the gastric fluid may influence the physiological effects of the ingested yeast. Almost all of the accumulated SAM in lyophilized SAM yeast had been released at zero time (Fig. 1A), suggesting that the cell walls of lyophilized SAM yeast are almost completely degraded by lyophilization and that lyophilized SAM yeast was digested efficiently in SGF. Stability in gastric fluid is also necessary for the SAM in lyophilized SAM yeast to be absorbed from the intestines and utilized in the body after feeding. SAM released from the lyophilized SAM yeast was stable in SGF for at least 2hrs (Fig. 1A), indicating that SAM derived from SAM yeast could reach the intestines and be absorbed. SAM was absorbed gastrointestinal after an oral administration, and an analysis of the biodistribution of 11C-SAM demonstrated the uptake of 11C-SAM in the kidney, small intestine, pancreas, adrenal gland, liver and spleen.

SAM yeast suppressed the ethanol-induced elevation of TG (a marker of steatosis; Fig. 4A) and ALT (a marker of liver injury; Fig. 4B), demonstrating that SAM yeast suppressed acute alcoholic liver injury. The ingestion of a diet containing 0.25% sake yeast tended to suppress acute alcoholic liver injury, but the suppressive effect was much weaker than that of a diet containing 0.25% SAM yeast; the ethanol-induced increases in TG in the ethanol, 0.25% SAM yeast/ethanol, and 0.25% sake yeast/ethanol groups were 2.5-, 1.7-, and 2.2-fold, respectively (Fig. 4A); the ethanol-induced increases in ALT in the ethanol, 0.25% SAM yeast/ethanol, and 0.25% sake yeast/ethanol groups were 7.7-, 2.6-, and 4.1-fold, respectively (Fig. 4B). We have shown in a previous report that the ingestion of a diet containing 1% sake yeast suppressed acute alcoholic liver injury; the ethanol-induced increases in TG in the ethanol, 0.25% SAM yeast/ethanol, and 0.25% sake yeast/ethanol groups were 2.5-, 1.7-, and 2.2-fold, respectively (Fig. 4A); the ethanol-induced increases in ALT in the ethanol, 0.25% SAM yeast/ethanol, and 0.25% sake yeast/ethanol groups were 7.7-, 2.6-, and 4.1-fold, respectively (Fig. 4B). The suppression effect of a diet containing 1% sake yeast against acute alcoholic liver injury was nearly the same as that of a diet containing 0.25% SAM yeast, suggesting that SAM yeast could suppress alcoholic liver injury at a lower doses than sake yeast could. The physiological effects of brewer’s yeast at higher doses (e.g. 5–15%) have been examined previously. As shown here, lower doses of SAM yeast and sake yeast could also produce physiological effects.

SAM yeast repressed the ethanol-induced decrease in hepatic SAM level (Fig. 5). Ethanol administration causes abnormal methionine metabolism in the liver by inhibiting the activity of methionine synthase, which is involved in the conversion of Hcy to methionine, and the formation of SAM from methionine catalyzed by methionine adenosyltransferase is decreased. In the acute alcoholic liver injury model we used, the administration of ethanol resulted in a decrease in SAM (Fig. 5), indicating that ethanol induced abnormal methionine metabolism in the liver. These results suggest that SAM yeast suppressed the ethanol-induced disorder of methionine metabolism and seems to have been involved in the suppression of acute alcoholic liver injury. We have demonstrated that a diet containing 1% sake yeast repressed both the ethanol-induced elevation of TG and ALT and the ethanol-induced reduction of the hepatic SAM level. Even though a diet containing 0.25% sake yeast repressed the ethanol-induced reduction of hepatic SAM level (Fig. 5), sake yeast could not fully repress the ethanol-induced elevations of TG and ALT (Figs. 4A and B). Improvement of the disorder of methionine metabolism after an ethanol treatment is thought to be one of the reasons for the suppression of alcoholic liver injury.

A distinctive nutritional characteristic of SAM yeast is the content of SAM (Table 2), indicating that SAM was involved in the suppression of acute alcoholic liver injury by SAM yeast. A diet containing 0.25% SAM yeast or 0.25% sake yeast respectively contains 19.5 mg or 0.113 mg of SAM per 100 g of the experimental diet. Estimating from Tables 2 and 3, mice fed on a diet containing 0.25% SAM yeast and 0.25% sake yeast are assumed to have ingested 21.3 mg and 131 μg of SAM per kg of BW daily. An intraperitoneal administration of SAM (50 mg/kg BW) once a day for three days has protected mice against acute alcoholic liver injury. The amount of SAM ingested by the mice fed the SAM yeast seems to have been enough to suppress acute alcoholic liver injury. In fact, SAM yeast could suppress acute alcoholic liver injury at a lower dose than sake yeast could, suggesting that the accumulated SAM in SAM yeast partly enhanced the suppressive effect. However the mice fed with SAM yeast consumed 163 times as much SAM daily compared to the mice fed with sake yeast, both SAM yeast and sake yeast could suppress the ethanol-induced decrease in hepatic SAM level (Fig. 5). The hepatic SAM level seems to have been kept constant through the methionine metabolic pathway.

Another distinctive nutritional characteristic of SAM yeast is its content of folate (Table 2). The folate levels in SAM yeast and sake yeast were 26.6-fold and 8.9-fold higher than that in X2180-1A (Table 2). The folate level in the control diet was estimated at 20.8 μg/100 g, and the control mice ingested 21.5 μg of folate per kg of BW daily, as estimated from Tables 1 and 3. The addition of 0.25% SAM yeast or sake yeast to the control diet increased the total folate level to 38.2 and 26.7 μg/100 g of experimental diet, respectively. The mice fed the SAM yeast or sake yeast ingested 42.3 μg or 30.9 μg of folate per kg of BW daily, as estimated from Tables 1 and 3. It has been suggested that the alcohol-induced increase in the plasma Hcy level was suppressed by sufficient folate intake in humans. Folate in its 5-methyltetrahydrofolate form is a cofactor in the con-
version of Hcy to methionine, and enhancement of this conversion may result in a decrease in Hcy and an increase in SAM. SAM yeast and sake yeast suppressed the ethanol-induced reduction of hepatic SAM level (Fig. 5). Folate in SAM yeast and sake yeast may affect methionine metabolism and partly account for the suppression of alcoholic liver injury. However, it is difficult to identify the components in SAM yeast and sake yeast responsible for the suppression of acute alcoholic liver injury, because the yeasts consist of many components, and several components may work synergistically.

In summary, this study shows that SAM yeast prevents acute alcoholic liver injury by improving methionine metabolism in the liver. The effect may partly depend on SAM and folate, which are present in SAM yeast. Compared with sake yeast, SAM yeast showed enhanced suppression of acute alcoholic liver injury in mice.

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References