Brewer’s and baker’s yeasts appear to have components that protect from liver injury. Whether sake yeast, Saccharomyces cerevisiae Kyokai no. 9, also has a hepatoprotective effect has not been examined. Here we show that sake yeast suppresses acute alcoholic liver injury in mice. Male C57BL/6 mice that had been fed a diet containing 1% sake yeast for two weeks received three doses of ethanol (5 g/kg BW). In the mice fed sake yeast, ethanol-induced increases in triglyceride (TG) and glutamate pyruvate transaminase (GPT) were significantly attenuated and hepatic steatosis was improved. In addition, sake yeast-fed mice showed a smaller decrease in hepatic S-adenosylmethionine (SAM) level and a smaller increase in plasma homocysteine (Hcy) level after ethanol treatment than the control mice, suggesting that a disorder of methionine metabolism in the liver caused by ethanol was relieved by sake yeast. These results indicate that sake yeast protects against alcoholic liver injury through maintenance of methionine metabolism in the liver.

Key words: Saccharomyces cerevisiae; sake yeast; liver injury; alcohol; methionine metabolism

The physiological effects of brewer’s yeast and its components in mammals have been well studied. For example, brewer’s yeast cell wall reduces serum lipid levels in rats, and improves defection by contributing to the fermentation ability, water holding capacity, and swelling force of the large intestine. To understand the gene expression of Saccharomyces bayanus as a lager brewer’s yeast during beer fermentation, sequencing of the whole genome was performed. This provided further information about the physiological effects of brewer’s yeast as well as the brewing of beer.

On the other hand, there have been few studies of the physiological effects of sake yeast, Saccharomyces cerevisiae Kyokai yeast, which is used in sake (Japanese rice wine) brewing. Manabe et al. reported that both sake yeast and sake lees, the leftovers of sake brewing, increase spontaneous locomotive activity in rats. They proposed that the effect of sake lees partly depends on sake yeast. The sake lees contain rice components that are not assimilated by sake yeast and sake koji, Aspergillus oryzae, components of sake yeast and sake koji, and their metabolites. Therefore, to understand the physiological effects of sake yeast may help to understand the physiological effects of sake lees and to find new uses for them.

A distinctive characteristic of sake yeast is that it is able to accumulate higher levels of S-adenosylmethionine (SAM) intracellularly than other yeasts, bacteria, molds, and some other microorganisms. SAM functions as a major methyl group donor in transmethylation of proteins, nucleic acids, polysaccharides, phospholipids, and fatty acids and as a precursor of glutathione. It has been proposed as a chemotherapeutic agent for alcoholic liver disease, depression, osteoarthritis and Alzheimer’s disease. Another distinctive characteristic of sake yeast is that it has a higher ethanol tolerance than any other yeast strain, due to its higher content of ergosterol, which is the predominant sterol in Saccharomyces cerevisiae. Ergosterol is a precursor of vitamin D, a fat-soluble vitamin that promotes osteoclastogenesis through enhancement of the absorption of calcium and phosphoric acid. Such constituents probably have physiological applications.

Several yeasts have components, such as amino acids and glutathione, that suppress liver injury induced galactosamine and ethanol. Accordingly, yeasts and yeast derivatives are promoted as health foods, even though few in vivo studies on liver injury have been conducted. Here we tried to analyze the effect of sake yeast on acute alcoholic liver injury in mice. Ethanol administration induces abnormal methionine metabolism in the liver. The first step in methionine metabolism is the formation of SAM catalyzed by methionine adenosyl transferase. The second step is the formation of S-adenosylhomocysteine by a transmethylation reaction. The third step is the formation of homocysteine (Hcy) catalyzed by SAH hydrolase. Hcy is further catalyzed in two ways: the regeneration of methionine catalyzed by methionine synthase, which requires folate and vitamin B12, and the formation of cystathionine catalyzed by cystathionine-β-synthase, which requires vitamin B6 as a cofactor. Cystathionine...
is converted into glutathione after three steps of reaction. Ethanol administration inhibits the activity of methionine synthase and results in an increase in Hcy and a decrease in SAM.\textsuperscript{15–19} We also examined the effect of sake yeast on methionine metabolism after ethanol treatment, and compared the contents of water-soluble vitamins such as folate and vitamin B6 that are required for methionine metabolism between sake yeast and a laboratory yeast strain X2180-1A.

**Materials and Methods**

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

**Measurement of water-soluble vitamins.** Water-soluble vitamins, vitamin B6 and folate, in yeast cells were measured by the SRL Food Analysis Service (Tokyo) by a microorganism assay system. Cultivated yeast cells were washed three times with sterilized distilled water and analyzed.

**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). The animals had free access to food and water. All animals received humane care, as outlined in "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 d, the mice were fed a control diet with or without 1% sake yeast (Table 1) for 14 d. Sake yeast was prepared as described above, and added to the control diet, replacing an equal amount of casein (Table 1).

**Results**

**Induction of acute alcoholic liver injury.** The binge drinking mouse model developed by Carson and Pruett\textsuperscript{20} was utilized for induction of acute alcoholic liver injury. After adaptation for 7 d, mice were divided into three groups: control, ethanol treatment, and sake yeast/ethanol treatment. The control and ethanol treatment groups were fed a control diet for 14 d, and the sake yeast/ethanol treatment group was fed a diet containing 1% sake yeast for 14 d. The mice in the ethanol and sake yeast/ethanol groups received ethanol (5 g/kg BW) by gavage every 12 h for a total of three doses. The mice in the control group received an isocaloric maltose solution instead of ethanol. At 12 h after the final ethanol dose, the mice were killed by decapitation to obtain blood and liver. Blood plasma was separated from heparinized whole blood by centrifugation. The plasma and liver obtained were stored at −80°C until analysis.

**Biochemical analysis of plasma.** The activity of alanine aminotransferase (ALT/GPT, EC 1.1.1.27) and the level of triglyceride (TG) in the plasma were measured colorimetrically by a commercial assay system, Fuji DRICHEM (Fuji Film Medical, Tokyo). The level of total homocysteine (Hcy) in the plasma was assayed by Homocysteine Microtiter Plate Assay (Diazyme, San Diego, CA).

**Histopathological examination.** Liver tissues were cut and fixed with 4% paraformaldehyde/PBS. The tissue slices were embedded in paraffin. Tissue sections of 5 μm were stained with hematoxylin and eosin (HE) and observed under a light microscope.

**Statistical analysis.** Data were expressed as means ± SEM. Statistical analysis was performed by one-way ANOVA, and the differences between the means were tested by the Tukey-Kramer test when the F-value was significant. A *p*-value of 0.05 or less was considered significant.

**Table 1.** Composition of Experimental Diets (w/w %)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>1% Sake yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>51.5</td>
<td>51.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Minerals</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamins</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sake yeast</td>
<td>—</td>
<td>1</td>
</tr>
</tbody>
</table>

Control diet was based on AIN-76 (Oriental Yeast, Tokyo).
Effects of Sake Yeast on Plasma TG and GPT Levels in Mice with Acute Alcoholic Liver Injury.
Mice were fed a control or a sake yeast-containing diet for 14 d as described in “Materials and Methods.” The mice in the EtOH and sake yeast/EtOH groups were given 5 g/kg of ethanol every 12 h three times by gavage, and those in the control group were given maltose solution instead of alcohol. Blood was collected 12 h after the third administration of ethanol. Values are mean ± SEM (n = 8). Values with different letters are significantly different at p < 0.05 by the Tukey-Kramer test.

3.9-fold respectively (Fig. 1A and B), whereas ethanol treatment of sake yeast-fed mice did not significantly affect TG or GPT levels (Fig. 1A and B). These results indicate that ingestion of sake yeast suppresses ethanol-induced elevation of TG and GPT.

Suppressive effect of sake yeast on acute ethanol-induced fat accumulation in liver
A fatty liver is one of the earliest consequences of alcohol abuse. Livers were normal both in the mice fed a control (Fig. 2A) and in those fed a sake yeast-containing diet (Fig. 2C) without ethanol treatment. Ethanol treatment caused the livers to become hypertrophic and turn white because of fatty accumulation (Fig. 2B). However, ethanol treatment had a smaller effect on the livers of mice given the sake yeast diet (Fig. 2D). Without ethanol treatment, liver sections were normal in the control mice (Fig. 2E) and sake yeast-fed mice (Fig. 2G). Ethanol treatment caused swelling and an increase in fat vacuoles in the hepatocytes (Fig. 2F), suggesting that alcohol-induced fat accumulation occurred in the liver. In the sake yeast/ethanol group, the size of the fat vacuoles was much smaller in the hepatocytes, and hepatic steatosis was attenuated (Fig. 2H). These results support the thesis that ethanol-induced steatosis of the hepatocytes is less severe in mice fed sake yeast.

Prevention of ethanol-induced Hcy elevation by sake yeast
The sake yeast diet did not affect the plasma Hcy level (Fig. 3). Chronic ethanol administration in mice increases hepatic and plasma Hcy levels by inhibition of the conversion of Hcy to methionine. In the present study, acute ethanol administration increased the total Hcy level 3.4-fold in the control mice, whereas it increased the Hcy level by only 1.2-fold in the sake yeast-fed mice (Fig. 3), indicating that ethanol-induced elevation of the Hcy level is suppressed by sake yeast.

Sake yeast increases hepatic SAM level and suppresses ethanol-induced decrease in hepatic SAM level
The sake yeast diet tends to increase the hepatic SAM level by 1.2-fold (Fig. 4). Ethanol treatment decreased the hepatic SAM level by 51.5% in the control mice, whereas it did not significantly change the SAM level in the sake yeast-fed mice (Fig. 3), indicating that sake yeast increases the hepatic SAM level and suppresses the reduction of the hepatic SAM level caused by ethanol treatment.

Contents of water-soluble vitamins related to methionine metabolism in sake yeast
The content of folate, a methionine-related vitamin, was 8.9-fold greater in sake yeast compared with X2180-1A (Table 3). On the other hand, the content of vitamin B6, another methionine-related vitamin, was not different between two strains (Table 3).

Discussion
First, sake yeast inhibits ethanol-induced increases in TG (a marker of steatosis, Fig. 1A) and GPT (a marker of liver injury, Fig. 1B). These results were supported by macroscopic and microscopic observations that revealed that sake yeast attenuates fatty accumulation in the liver (Fig. 2A and B). Second, the suppressive effect of sake yeast on acute alcoholic liver injury is accompanied by suppression of the ethanol-induced decrease in hepatic SAM level (Fig. 4) and the ethanol-induced increase in plasma Hcy level (Fig. 3), suggesting that the abnormal methionine metabolism due to ethanol treatment improves. Chronic ethanol consumption alters methionine metabolism in the liver by inhibiting the activity of methionine synthase, which is responsible for conversion of Hcy to methionine. Inhibition of methionine synthase results in a decrease in
the hepatic level of SAM and increased generation of Hcy,\textsuperscript{15-19} which is released from the liver and causes abnormal methionine metabolism. In the acute alcoholic liver injury model we used,\textsuperscript{20} we confirmed a decrease in the hepatic SAM level (Fig. 4) and an increase in the plasma Hcy level (Fig. 3), suggesting that methionine metabolism in the liver was in disorder. Accordingly, sake yeast prevents the ethanol-induced disorder of methionine metabolism by restoring the hepatic SAM level and by preventing an increase in the Hcy level.

We showed that the addition of 1\% sake yeast suppresses ethanol-induced liver injury. The physiological effects of Brewer’s yeast were observed previously at higher doses. For example, feeding of a diet containing 5\% Brewer’s yeast prevented obesity in mice,\textsuperscript{23} and that containing 15\% Brewer’s yeast influenced the intestinal immune system and suppressed type II collagen-induced arthritis in mice.\textsuperscript{24} As shown here, lower doses of yeast can show physiological effects.

It appears that several hepatoprotective components of sake yeast work together to suppress alcoholic liver injury because yeasts have several kinds of hepatoprotective components. Sake yeast accumulates SAM,\textsuperscript{5,6} which has been shown to protect against liver injury. The SAM level of sake yeast (0.45 mg/g of dry cell weight) was higher than that in X2180-1A (0.19 mg/g of dry cell weight), but this level was not as high as previously found,\textsuperscript{5,6} possibly because the culture conditions used in this study were not suitable for accumulating SAM. Administration of SAM (50 mg/kg BW) has been shown to protect against acute alcoholic liver injury in mice.\textsuperscript{25} Therefore, the SAM content in sake yeast may not be enough to suppress acute alcoholic liver injury under the conditions tested here. Folate, which is present at high levels in sake yeast (Table 3), appears to be one of the more likely candidates to suppress acute alcoholic liver injury. The
folic acid level of a control diet is estimated to 20.8 μg/100 g, and the addition of 1% sake yeast increases the folic acid level of the diet to 44.2 μg/100 g. Folate in its 5-methyltetrahydrofolate form is required for the recycling of methionine from Hcy. Therefore, folate may have affected the plasma Hcy and hepatic SAM levels in the mice fed sake yeast. In addition, sake yeast increased the hepatic SAM level and protected against liver injury (Figs. 1 and 4). The effect of sake yeast on the hepatic SAM level is also expected to depend on folate. Folate ingestion probably causes an increase in regeneration of methionine, which causes an increase in hepatic SAM.

Oxidative stress is also involved in alcoholic liver injury, and antioxidants that decrease free radical formation can suppress alcoholic liver injury. Antioxidants in green tea extract have been proposed to prevent acute alcoholic liver injury. Glutathione is an antioxidant present at high levels in yeasts. In sake yeast and X2180-1A, we measured glutathione levels of 13.7 mg/g of dry cell weight and 10.6 mg/g of dry cell weight respectively. Mitochondrial glutathione is depleted after ethanol treatment. The glutathione in sake yeast probably increases the hepatic glutathione level and therefore protects against oxidative stress after ethanol treatment. Moreover, in alcoholic liver injury, the free radicals formed lead to increased formation of proinflammatory cytokines such as TNFα, which promote inflammation in the liver, and suppression of TNFα production by Hydrgaeus Dulcis Folium (ama- cha) has been shown to prevent acute alcoholic liver injury. Glycine also suppresses TNFα production in d-galactosamine-sensitized rats and is likely to be effective in alcoholic liver injury. Yeasts have many amino acids. The glycine levels of sake yeast and X2180-1A were 28.5 mg/g of dry cell weight and 21.4 mg/g of dry cell weight respectively. As shown above, sake yeast has several constituents that inhibit oxidative stress and cytokine production, which are probably related to suppression of alcoholic liver injury.

In summary, this study indicates that sake yeast prevents acute alcoholic liver injury by improving methionine metabolism in the liver. The effect may depend on several constituents of sake yeast. Recently, a method of isolating a SAM-accumulating yeast was reported. The new method may lead to higher production of SAM in sake yeast, which may reveal new physiological functions of sake yeast.

Acknowledgment

This study was supported by a grant from the Japan Sake Brewer’s Association. We thank Ms. K. Isobe for technical assistance.

References

Suppression of Alcoholic Liver Injury by Sake Yeast


