Note

Increase in Spontaneous Locomotive Activity in Rats Fed Diets Containing Sake Lees or Sake Yeast

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The process of Sake making generates a great deal of secondary product called Sake lees. Part of the Sake lees is disposed of as waste, so finding ways to recycle it are a matter of concern. Because Sake lees contains many nutritious substances derived from both rice and Sake yeast, we studied the physiological effects of these materials on rats. Sprague-Dawley rats were fed one of three experimental diets for two weeks. Rats fed diets containing either 20% Sake lees or 2% Sake yeast (Kyokai 9 yeast) both had higher spontaneous locomotive activity than rats fed a control casein diet. Body weight and food consumption were not different among the three groups. These results suggest that Sake lees and Sake yeast have a reinforcing effect on spontaneous locomotive activity.

Keywords: Sake lees, Saccharomyces cerevisiae, Sake yeast, spontaneous locomotive activity.

Introduction

Sake (Japanese rice wine) is a traditional Japanese alcohol beverage. It is made with Saccharomyces cerevisiae Kyokai yeast (Sake yeast). The main ingredients of Sake are yeast and rice and koji. After making Sake, there is a great deal of leftover material, called Sake lees. The demand for Sake lees is limited, so some of it ends up as industrial waste. Uses for Sake lees might increase if its benefits were better known, since the beneficial health effects of beer yeast are well known and used as a dietary supplement (Kato 1998, Nakamura 2001). Sake lees is expected to have some health benefits because it contains protein, carbohydrate and many vitamins. In addition, Sake yeast produce more ergosterol and S-adenosyl methionine (SAM) than normal S. cerevisiae (Shiozaki et al. 1984, 1989). SAM is a major biological methyl donor, which was reported to have many key functions. In human SAM stimulates the synthesis of proteoglycans by articular chondrocytes, result in some improvement in arthritis (Echols et al. 2000). Supplementation of SAM to patients with alcoholic liver disease improved the mitochondrial GSH levels, resulting in attenuation of ethanol-induced oxidative stress and liver injury (Echols et al. 2000, Lieber 2002a, 2002b). In the central nervous system, the methyl group of SAM is transferred to a neurotransmitter such as norepinephrine, dopamine or serotonin, which improves depression (Echols et al. 2000). In fact, SAM is used as a nutritional supplements in the United States. Although the effects of SAM have been well studied, studies of the effects of Sake lees or Sake yeast are quite limited (Saito et al. 1994a, 1994b; Tsutsui et al. 1998). If SAM in Sake yeast influences the central nervous system, it is expected that administration of Sake lees or Sake yeast has some activity effect. Therefore, this study was undertaken to assess the chronic effect of eating a diet containing Sake yeast or Sake lees on spontaneous activity in rats.

Materials and Methods

Eight-week-old male Sprague-Dawley (SD) rats (Charles River Japan, Yokohama, Japan) were maintained under controlled conditions (22 ± 0.5°C temperature, 60% humidity, 12 hr light-dark cycle, lights off 12:00 and on 0:00). The rats were housed individually in plastic cages (33 x 23 x 12 cm). All animals received humane care as outlined in the Guide for the Care and Use of Laboratory Animals (Animal Care Committee, National Research Institute of Brewing). Rats were weighed every other day. They were first given a commercial diet (CLEA Rodent Diet CE-2, Clea Japan Inc., Tokyo) for 1 wk. To assess their spontaneous activity, we used an voluntary exercise wheel (Shinano Co., Ltd., Tokyo) with a computer output that counts wheel rotations. Each rat was moved into a wire-mesh cage (13 × 40 × 15 cm) with a voluntary exercise wheel for two hours per day. Taking into consideration their activity time, a running exercise was performed at the same time (14:00–16:00 or 16:00–18:00) throughout the experimental period. During this running time they had no access to food. This was repeated every day for a week to habituate the rats to the wheel. The diets were then switched from a
commercial diet to a casein based diet (Table 1). During this time (second week) the animals were habituated to eat the casein diet. The average number of rotations for the last three days in the second week was used as the basal rotation. Then the rats were divided into three groups, each receiving a different diet: a casein diet (control group), a 20% Sake lees diet (Sake lees group), and a 2% Kyokai 9 yeast diet (Sake yeast group). Sake lees diets and Sake yeast diets were prepared so that they were equal in protein and carbohydrate to the casein diet. A 20% content of Sake lees was used based on previous studies which reported this to be effective in decreasing serum cholesterol (Mochida et al. 2000). Sake lees were prepared as described previously (Tsutsui et al. 1998). Sake yeast was cultured in YPD medium at 30°C for three days using a 90-L jar fermenter. Animals were given free access to each diet for 2 weeks (third week and fourth week) and were run in the exercise wheel for 2 hr per day as before. Food consumption was measured every day and converted from dry weight to calories. Spontaneous activity was expressed as the ratio of the average number of rotations in a two-day period divided by the average number of rotations in the last 3 days of the basal training period.

### Table 1. Composition of experimental diets (w/w %)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>20% Sake lees</th>
<th>2% Sake yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>25</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>51.5</td>
<td>36.5</td>
<td>51.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Minerals</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamins</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sake lees</td>
<td>-</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Sake yeast</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Calories</td>
<td>379.3</td>
<td>341.7</td>
<td>371.3</td>
</tr>
</tbody>
</table>

Control diet was based on AIN-76 diet likeness of Oriental Yeast Co., Ltd. (Tokyo).

### Results and Discussion

The body weight and the amount of calories consumed did not differ among the three groups (Fig. 1(a) and (b)). The feces showed no apparent symptoms of diarrhea due to the ingestion of yeast. The average basal rotation also was not different among the three groups (casein group 1193 ± 175.1, Sake lees group 983 ± 178.0, Sake yeast group 986 ± 140.1). Although the body weight, food intake and basal activity of the groups were similar, the spontaneous activity of rats fed the 2% Sake yeast diets increased significantly on the 14th day (Fig. 2, *; P < 0.01 from casein group, Tukey test). Values on x-axis refer to average activity in two day intervals. Values are means ± SEM (n; casein = 11, Sake yeast = 12, Sake lees = 10). These results suggest that feeding a regular diet of Sake yeast or Sake lees increases the spontaneous activity of the animals. Although Sake lees is composed of 30–40% Sake yeast on a dry weight basis, the increase in effect of spontaneous activity in this group was not stronger than that in the Sake yeast group. The reason for this small increases
in spontaneous activity in the Sake lees group is uncertain. One possibility is that it was due to the state of preservation of the lees. Sake lees is obtained by brewing from liquefied rice, therefore it contains not only Sake yeast but also protein, sugar and some vitamins. The effect of sake yeast in Sake lees might not exert any direct effect. Although it is unclear which components of the diets were responsible for an increase in the spontaneous activity, the fact that control yeast had no effect suggests that a characteristic component in Sake yeast such as SAM is one candidate. Shiozaki et al. (1984) reported that Sake yeast exhibited the highest accumulation of SAM. In agreement with their finding, we confirmed there was a high accumulation of SAM in Sake yeast (3–4 mg/g dry cell). From our measurement, Sake lees also contained SAM derived from Sake yeast at high concentration (unpublished data).

SAM is required for methylation reactions in the nervous system. A low level of SAM in cerebrospinal fluid (CSF) is reported to be related to depression (Echols et al. 2000). Oral administration of SAM crossed the blood-brain barrier and influenced monoamine metabolism (Bottiglieri et al. 1984). Intravenous or oral administration of SAM was shown to increase its level in CSF (Bottiglieri et al. 1990). In human, a usual oral dosage of SAM for the treatment of depression is SAM200–800 mg (Echols et al. 2000). If this dosage has an effect on the neural processes, SAM content of about 20 mg in a Sake yeast diet is enough to affect its activity in rats. These results raise the possibility that SAM in a Sake yeast diet increases spontaneous locomotive activity through its positive effects on the nervous system. However, it does not rule out the possibility that additional substances other than SAM in Sake yeast affect this activity. Further studies are needed to determine the effect of Sake yeast on the spontaneous locomotive activity. In a previous study of rats fed a high cholesterol diet containing 0, 10 or 20% Sake lees powder, animals fed the Sake lees-supplemented diets had significantly lower serum cholesterol (Mochida et al. 2000). A diet containing 50% Sake lees powder was also shown to have a hypolipidemic effect on rats without affecting growth rate (Tsutsui et al. 1998). Furthermore, Sake lees contains antihypertensive peptide (Saito et al. 1994a, 1994b). These results, together with our findings of increased spontaneous activity in rats fed Sake lees, suggest that it and Sake yeast can be recycled as new functional foods. The recycling of Sake lees will also reduce industrial waste.

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References