Combined Effect of Lysed Enterococcus faecalis FK-23 and Fruits of Citrus unshiu on Contact Dermatitis in Mice

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The aim of the present study was to examine the combined effect of oral administration of lysozyme-treated Enterococcus faecalis FK-23 (LFK) and fruits of Citrus unshiu (CU) on the allergic contact dermatitis. ICR mice were orally administered with LFK (50, 500, and 1500 mg/kg body weight/day) and/or CU (50 and 200 mg/kg body weight/day) for 8 days. Mice were transdermally sensitized with 7% picryl chloride to the abdomen at one day after the first administration, and were elicited with 1% picryl chloride to each side of the ears seven days after the sensitization. At twenty-four hours after the elicitation, the ear thickness and the organ weight (adrenal gland, thymus and spleen) were recorded. Oral administration of LFK or CU alone significantly inhibited the picryl chloride-induced ear swelling compared to control group. Moreover, a marked additive effect was observed when LFK and CU were orally administered in combination. There was no significant change in the organ weight after the administration of LFK and/or CU. These results indicated that the combined administration of LFK and CU ameliorate type IV allergic symptoms without systemic side effects.

Key words: contact dermatitis/ type IV allergy/ Enterococcus faecalis/ Citrus unshiu

INTRODUCTION

Hypersensitivity reactions can be divided into four types based on the mechanism and the reaction time: type I (IgE-mediated hypersensitivity); type II (cytotoxic hypersensitivity); type III (immune complex hypersensitivity) and type IV (cell-mediated hypersensitivity) (Cruse JM et al., 2010). Allergic contact dermatitis, which is a clinical term used to describe an inflammatory reaction in the skin, is caused by type IV delayed type hypersensitivity responses to antigens (Cruse JM et al., 2010). This response is initiated by not antibodies as in type I, type II, type III responses but T-lymphocytes. The development of allergic contact dermatitis results in the significant reduction in quality of life and the economic loss (Kaplan DH et al., 2013). The discovery and development of anti-allergic agents without side effects are required to control the allergic symptoms.

Lactic acid bacteria have attracted considerable attention as not only the probiotic effect but also the treatment options for the development of type I allergic symptoms such as allergic rhinitis, asthma and conjunctivitis. Certain lactic acid bacteria including lysozyme-treated Enterococcus faecalis FK-23 (LFK) reduce the nasal symptoms by the promotion of regulatory T cells (Zhu L et al., 2012) and suppress allergic asthma airway inflammations by the attenuation of Th17 cell development (Zhang B et al., 2012, Jan RL et al., 2012). However, there have been no reports on the effect of lactic acid bacteria in type IV allergic symptoms. Segawa et al. previously demonstrated that Lactobacillus brevis SBC8803 ameliorates the development of
dermatitis induced by the repeated treatment of picryl chloride (Segawa S et al., 2008). However, this murine dermatitis model does not represent the type IV hypersensitivity, since it is known that the allergic reaction shifts from the delayed-type to immediate-type hypersensitivity by the repeated treatment of picryl chloride (Kitagaki H et al., 1995).

Previous studies show that fruits of Citrus unshiu (CU) have various biological and pharmacological activities including anti-allergic (Kubo M et al., 1989, Park SH, 2005), anti-viral (Suzuki M et al., 2005), and anti-diabetic effect (Park HJ et al., 2012). We previously reported that the extract of CU has anti-allergic properties in the picryl chloride-induced contact dermatitis as type IV allergic model in mice (Kubo M et al., 1989).

We have studied the anti-allergic effects of lactic acid bacteria and citrus fruits as above. These results implied the possibility of the combined effect of these on allergic responses. In this study, we investigated the effect of oral administration of LFK and CU on the development of the contact dermatitis, which was induced by the topical application of picryl chloride in mice.

Materials and methods

LFK preparation.

LFK was prepared as described previously (Fukada K et al., 2013). Briefly, E. faecalis strain FK-23 was cultured in broth medium containing 2.5% of glucose, 1.4% of yeast extract, 0.8% of peptone, and 4.4% of K2HPO4 for 18h at 37°C, and the cultures were harvested by centrifugation. After washing with distilled water, the bacteria were treated with lysozyme, and then the reaction mixture was heated to 105°C for 10 min before lyophilization.

CU preparation.

Unripe fruit of C. unshiu were air-dried at 50°C for 48 h in an automatic air-drying apparatus (Vianove Inc., Japan), and powdered. The powder (300 g) was extracted with 50% ethanol (3 liter) for 2 h under reflux. The extract was evaporated under reduced pressure and then lyophilized to give the 50% ethanol extract in 24.5% yield.

Mice and oral administration

Female ICR mice were purchased from Japan SLC Inc. Mice were housed in a conventional air-conditioned room (12 h light/dark cycles) with free access to standard diet (CE-2; CLEA Japan) and tap water. LFK and/or CU dissolved in 0.2% sodium carboxymethylcellulose (Na-CMC) were orally administered to mice (6 weeks old, 27—29 g) using a feeding needle once a day from day 0 to day 7. Prednisolone (Nacalai Tesque, Japan) dissolved in 0.2% Na-CMC was orally administered once a day from day 2 to day 7. All experimental protocols were approved by the Committee for the Care and Use of Laboratory Animals at Kinki University and were accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Picryl chloride-induced contact dermatitis

Contact dermatitis was induced by picryl chloride as described previously (Asherson GL et al., 1968) with some modifications of the protocol. Mice were sensitized by the transdermal administration of 7% picryl chloride (0.1 mL) dissolved in ethanol to the shaved abdomen on day 1. Seven days after the first sensitization, 0.02 mL of 1% picryl chloride dissolved in olive oil was applied to each side of the ears. Ear thicknesses before and twenty-four hours after the elicitation were measured. After euthanasia by cervical dislocation, adrenal gland, thymus, and spleen were removed and the organ weight was recorded.

Statistical analysis

All values are expressed as mean ± S.E. Statistical evaluation of the results in the ear thickness and the organ weight was performed by Bonferroni/Dunn's method. All differences were considered significant at p<0.05.

Results and discussion

To investigate the combined effect of LFK and CU on the contact dermatitis, mice were sensitized and elicited with picryl chloride and the ear thickness was measured after the elicitation (Fig. 1 and Table 1). Oral administration of LFK showed the suppressing tendency of ear swelling at 50 and 500 mg LFK/kg and the significant suppression of it (27.7% inhibition) at 1,500 mg LFK/kg compared to control mice which were sensitized and elicited by picryl chloride. Oral administration of CU significantly inhibited the ear swelling at 50 and 200 mg CU/kg (25.0 and 36.2% inhibition, respectively) compared to control...
mice. Furthermore, a marked additive effect was observed when LFK and CU were orally administered in combination compared to LFK- or CU-administered group (Table 1).

Table 1  Effects of LFK and CU on ear swelling in picryl chloride-induced contact dermatitis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>n (mice)</th>
<th>Swelling (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>-</td>
<td>10</td>
<td>68.7 ±1.1</td>
<td>-</td>
</tr>
<tr>
<td>LFK</td>
<td>50</td>
<td>10</td>
<td>66.9 ±0.8</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>10</td>
<td>56.4 ±1.1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>9</td>
<td>49.7 ±1.8</td>
<td>27.7</td>
</tr>
<tr>
<td>CU</td>
<td>50</td>
<td>9</td>
<td>51.5 ±0.6</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>10</td>
<td>43.8 ±1.5</td>
<td>36.2</td>
</tr>
<tr>
<td>LFK+CU</td>
<td>50+50</td>
<td>11</td>
<td>50.0 ±1.8</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>50+200</td>
<td>11</td>
<td>40.2 ±2.7</td>
<td>41.5</td>
</tr>
<tr>
<td></td>
<td>500+50</td>
<td>11</td>
<td>48.4 ±2.2</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td>500+200</td>
<td>10</td>
<td>37.4 ±2.3</td>
<td>45.6</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>10</td>
<td>10</td>
<td>25.3 ±1.8</td>
<td>63.2</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.

**: Significantly different from control group at P<0.01

In addition, in the previous reports on the toxicological studies of LFK and CU, no significant dose-related changes are observed in the hematological and biochemical examination (Shimada T et al., 1997, Lyu JH et al., 2013). These results indicate that the oral administration of LFK and CU shows anti-contact dermatitis effect without systemic side effects.

Type IV allergy in which there are two stages (sensitization and an effector phase) is an inflammation caused by the interaction between an antigen and T cells (Cruse JM et al., 2010). An initial invasion of the antigen differentiates naïve T cells into memory T cells. In the secondary invasion, memory T cells immediately proliferate and differentiate into effector cells, and inflammation is evoked by the infiltration of the inflammatory cells into the site of allergen exposure. Previously, we reported that LFK suppresses the infiltration of inflammatory cells into the local sites by stabilizing the endothelial-epithelial permeability in influenza virus-infected and allergen-induced mice models (Shimada T et al., 2004, Fukada K et al., 2013). Therefore, in the same manner, LFK might protect the integrity of the epithelia-endothelial barrier and suppress the cellular infiltration including effector T cells in the ears, which causes edema. Further analyses including the release of chemical mediators that are involved in the increase of vascular permeability are required for comprehensive understanding of anti-allergic effect of LFK.

To elucidate the anti-inflammatory effect of CU in type IV allergy, we previously examined the effect in the acetic acid-induced vascular permeability and carrageenan-induced edema as acute inflammatory model (Kubo M et al., 1989). In these models, it is known that the injection of acetic acid or carrageenan induces chemical mediators such as prostaglandin E2, serotonin and histamine, leading to the increase in vascular permeability (Vinegar R et al., 1969, Deradet R et al., 1980). However, the positive effects on these by CU has not been obtained (Kubo M et al., 1989). These results indicate that CU has no effect on the release of chemical mediators involved in vascular permeability in type IV allergy. Citrus fruits including C. wushiu have numerous bioactive flavonoids. In particular, we identified hesperidin and nobletin as the active components in the extract of C. wushiu, and reported that these citrus flavonoids suppress the histamine release from mast cells (Matsuda H et al., 1991). Moreover, not only the natural flavonoids but also the flavonone glycosides that are activated by intestinal bacteria show anti-allergic effect (Fujita T et al., 2008, Park HJ et al., 2012). Therefore, one possibility is that these C. wushiu-derived flavonoids ameliorate the type IV allergy. Further examinations are required to identify the components of LFK and CU with anti-allergic effect and to understand the detailed action.
mechanism.

Table 3 Effects of LFK and CU on relative organ weight in picroxyl
chloride-induced contact dermatitis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>n (mice)</th>
<th>weight (% of body weight)</th>
<th>Aduenal gland</th>
<th>Thymus</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>-</td>
<td>9</td>
<td>0.18±0.001</td>
<td>0.19±0.01</td>
<td>0.48±0.03</td>
<td></td>
</tr>
<tr>
<td>LFK</td>
<td>50</td>
<td>10</td>
<td>0.19±0.001</td>
<td>0.20±0.03</td>
<td>0.45±0.03</td>
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</tr>
<tr>
<td></td>
<td>500</td>
<td>10</td>
<td>0.19±0.001</td>
<td>0.20±0.01</td>
<td>0.47±0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>9</td>
<td>0.18±0.001</td>
<td>0.18±0.01</td>
<td>0.48±0.03</td>
<td></td>
</tr>
<tr>
<td>CU</td>
<td>50</td>
<td>10</td>
<td>0.20±0.001</td>
<td>0.18±0.02</td>
<td>0.50±0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>10</td>
<td>0.18±0.001</td>
<td>0.20±0.02</td>
<td>0.43±0.02</td>
<td></td>
</tr>
<tr>
<td>LFK+CU</td>
<td>50+50</td>
<td>11</td>
<td>0.17±0.001</td>
<td>0.20±0.02</td>
<td>0.48±0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50+200</td>
<td>11</td>
<td>0.18±0.001</td>
<td>0.20±0.02</td>
<td>0.47±0.03</td>
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<td></td>
<td>500+50</td>
<td>10</td>
<td>0.18±0.001</td>
<td>0.17±0.02</td>
<td>0.50±0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500+200</td>
<td>10</td>
<td>0.17±0.001</td>
<td>0.20±0.02</td>
<td>0.46±0.02</td>
<td></td>
</tr>
<tr>
<td>Prednisolone</td>
<td>10</td>
<td>10</td>
<td>0.17±0.001</td>
<td>0.07±0.01</td>
<td>0.33±0.01 **</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. **Significantly different from control group at P<0.01

References
