Food Allergy and Liver Function—Preventive Effects of Drugs and Directory Fructooligosaccharides—

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[Background] Food allergies cause damage not only to the intestines of patients but to their livers as well, making it all the more important to discover what drugs are effective in preventing that damage. Accordingly, we studied the effectiveness of Chinese traditional medicine and FOS in helping to prevent damage caused by food allergies.

[Methods] (1) 13C-methacetine was used to study the relationship between liver damage and cases of food allergy presentation. (2) Chinese traditional medicine (Hochu-Ekito = TJ-41) was administered orally to mice twice a day for 2 weeks in a drug study of the food allergy model. (3) The effect of FOS on fecal organic acid production was observed in a study of FOS-treated mice.

[Results] (1) As a result of the 13CO2 excretion pattern, the relationship between liver function and food allergies was confirmed. (2) The serum ALT levels decreased in mice treated with TJ-41. (3) Short-chain fatty acids such as butyrate were significantly higher in the FOS-treated group than in the control group.

[Conclusion] Damage not only to the intestines but also the liver of food allergy patients was confirmed. TJ-41 and FOS were found to prevent this allergy-related damage, showing that FOS is very useful for allergic patients.

Key words: food allergy; liver function; atopic dermatitis; fructooligosaccharides

INTRODUCTION

The prevalence of food allergies in children is increasing because of the changes in their lifestyles and diets, which have become strongly Westernized. Especially in pediatrics, food allergies are significantly increasing. In Japan we conducted an immediate-type food allergy study, which began in 1998 and ended in 2000. In this study, the percentage of immediate-type food allergy patients below 6 years of age was 12.6%. Furthermore, the relationship between food and atopic dermatitis is a growing problem in young children.

Some patients with atopic dermatitis showed abnormal liver function (GOT & GPT). Why are the GOT and GPT levels increased in some patients who have been suffering from atopic dermatitis? In this paper, we would like to focus on the relationship between food allergies and liver function and suggest ways of preventing these abnormal conditions by using Chinese traditional medicine and fructooligosaccharides (FOS).

CASE PRESENTATION

Case: H.K., 14 months, Female. This patient has been suffering from atopic dermatitis since 5 months of age. Her GOT level was moderately high at 5 months, but it recovered to the normal range with a parallel improvement in her atopic dermatitis (Fig. 1).

We tried to determine whether this high GOT level came from the liver.

13C-methacetine excretion test. If the patient's liver function is normal, 13C-methacetine metabolized by P-450 enzyme in the liver and 13C is exhaled as 13CO2 (2). As shown in Fig. 2, the 13CO2 excretion demonstrated a low-peak level in patients with high GOT levels. On the other hand, a high and sharp peak appeared in patients with normal GOT levels. From this data, we suspected that patients with high GOT levels suffer from liver damage.

INTENTIONAL MICROFLORA IN ALLERGIC PATIENTS

The role of intestinal microflora in the maturation of the mucosal immune barrier is well recognized. This flora can potentiate the infant intestinal barrier by contributing a nonimmunological defense and by stimu-
Fig. 1. Case of a baby (HK, 14 months, female) with eczema whose serum GOT level was moderately high at 5 months of age but who recovered to the normal range with a parallel improvement in her eczema.

Fig. 2. Comparison of kinetics of excretion of $^{13}$CO$_2$ in breath following oral administration of $^{13}$C-methacetin to babies with high serum GOT (●), to the same babies following recovery of serum GOT (▲), and to babies with normal serum GOT (○).

lating and directing the development of the intestinal immune system.

This endogenous defense mechanism has a role in preventing allergic inflammation, especially during the weaning period.

The 52 subjects involved in the interventional bacterial flora study were infants and children from 3 months to 10 years of age. They were divided into allergic groups (n = 27) and nonallergic groups (n = 25).

The frequency of bifidobacteria was lower in the allergic group (67%) than in the nonallergic group (96%). From this data, intentional microflora is vital in protecting against allergic diseases.

**FOOD ALLERGY ANIMAL MODEL STUDY**

**Animal sensitization.** Six-week-old NC/Jic mice with high levels of serum IgE were obtained from Clea Co., Tokyo, Japan. The mice were housed at 23.5°C and 55% relative humidity. They were sensitized by 100 µg ovalbumin (OVA) interaperitoneally with 0.1 ml aluminum (Alum, Pierce Co., Rockford, IL) as an adjuvant, followed by an oral administration of 100 µg OVA five times a week. The nonsensitized control mice were treated similarly except that they received physiological saline instead of OVA solution.

One week after the final immunization, the mice were administered OVA (2 mg/0.1 ml) orally for an intestinal allergy challenge. Three hours after the oral challenge, they were killed and their liver and small intestine removed.

**Group division.** The mice were divided into three groups: Group 1 was a nonsensitized control group, group 2 was a sensitized control group treated with physiological saline, and group 3 received traditional Chinese medicine and FOS.

**Measurement of serum alanine aminotransferase (ALT).** The liver function is an index to evaluate hepatic injury. To evaluate liver function, we measured ALT in sera the mice through spectrophotometry (Amersham Pharmacia Biotech Co., Tokyo, Japan).

**Histological analysis of the small intestine.** Specimens were fixed in 10% buffered formalin and embedded in paraffin. Sections (4 µm) were stained with hematoxylin and eosin (H&E) and examined by light microscopy.

To identify mast cells, we examined sections stained...
Table 1. Hochu-ekki-to TJ-41.
A total of 7.5 g of this product contains 5.0 g of dried extract obtained from mixed raw herbs in the following ratio:

<table>
<thead>
<tr>
<th>Herb</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>JP Astragalus root</td>
<td>4.0 g</td>
</tr>
<tr>
<td>JP Atractyloides lancea rhizome</td>
<td>4.0 g</td>
</tr>
<tr>
<td>JP Ginseng root</td>
<td>4.0 g</td>
</tr>
<tr>
<td>JP Japanese angelica root</td>
<td>3.0 g</td>
</tr>
<tr>
<td>JP Bupleurum root</td>
<td>2.0 g</td>
</tr>
<tr>
<td>JP Jujube fruit</td>
<td>2.0 g</td>
</tr>
<tr>
<td>JP Citrus unshiu peel</td>
<td>2.0 g</td>
</tr>
<tr>
<td>JP Glycyrrhiza root</td>
<td>1.5 g</td>
</tr>
<tr>
<td>JP Cimicifuga rhizome</td>
<td>1.0 g</td>
</tr>
<tr>
<td>JP Ginger rhizome</td>
<td>0.5 g</td>
</tr>
</tbody>
</table>

Table 2. Serum ALT levels in mice with food-allergy-induced liver damage after treatment with TJ-41 or saline.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ALT (karmen unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitized</td>
<td>7</td>
<td>42.9 ± 7</td>
</tr>
<tr>
<td>TJ-41 (300 mg/kg/day)</td>
<td>6</td>
<td>8.6 ± 3.8*</td>
</tr>
<tr>
<td>TJ-41 (1000 mg/kg/day)</td>
<td>6</td>
<td>15.5 ± 2.74</td>
</tr>
</tbody>
</table>

*p < 0.05.

with toluidine blue. The identified mast cells were counted in 20 high-power fields (HPF) and expressed as the number of cells/HPF with mean ± SE calculation.

Measurement of IL-4, IL-6, and TNF-α. The immunohistochemical (streptavidin-peroxides) complex method was performed on formalim-fixed and paraffin-embedded sections for the cytokine expression. The monoclonal antibodies, rat antimouse IL-4mAb (IgG1, Enzyme Co.,) and TNF-α mAb (IgG1 Upstate Biotechnology Inc. Lake Placid, N.Y.) were used for immunostaining.

Drug study in food allergy model.

1. Chinese traditional medicine study
We used Hochu-ekki-to (TJ-41), which consists of two kinds of crude drugs shown in Table 1. TJ-41 was formed into pellets and diluted into 3 mg/100 ml of physiological saline. TJ-41 and normal saline were administered orally to the mice twice a day for 2 weeks, starting on the day of the fourth immunization.

2. FOS-treated study
Effects of FOS on fecal organic acid production. The concentrations of organic acid in the faces was analyzed quantitatively by HPLC.

Statistical analysis. The data shown as the mean ± SEM statistical analysis were obtaines by use of the Student’s t test.

RESULTS

TJ-41 Study
1. Evaluation of liver function: The levels of ALT in sensitized animals increased significantly compared to the nonsensitized animals. Moreover, the serum ALT levels decreased in mice treated with TJ-41 (Table 2).
2. Effect of TJ-41 on IL-4, IL-6, and TNF-α: The levels of expression of IL-4, IL-6, and TNF-α in livers treated with TJ-41 were lower than the levels measured in mice grown in normal saline (Fig. 3).

FOS Study
1. Histological analysis: After the challenge, mast cell numbers were higher in the group that received no FOS compared to the group that did.
2. Effects of FOS on fecal organic acid: short-chain fatty acids such as butyrate were significantly higher in...
### Table 3. Fecal organic acids in mice fed the control diet or the FOS diet.

<table>
<thead>
<tr>
<th>Organic acid</th>
<th>OVA-sensitized (µg/g feces)</th>
<th>OVA-sensitized fed with FOS (µg/g feces)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lactate</td>
<td>134 ± 50</td>
<td>986 ± 314*</td>
</tr>
<tr>
<td>Formate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acetate</td>
<td>776 ± 71</td>
<td>2218 ± 342*</td>
</tr>
<tr>
<td>Propionate</td>
<td>10 ± 6.5</td>
<td>317 ± 100*</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>839 ± 51</td>
<td>981 ± 148*</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0</td>
<td>187 ± 61*</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Valerate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Caproate</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

1Values are means ± SEM. *Significantly different from the OVA-sensitized mice (p < 0.01).

The FOS-treated group than in the control group (Table 3).

### DISCUSSION

Food allergies are becoming a big problem in pediatrics. Some food allergy patients’ ALT levels were high. We conducted a breath test with methacetine labeled with the stable 13C-isotope. As a result of the 13CO2 excretion pattern, the relationship between liver functions and food allergies was determined.

The results indicated an abnormal 13CO2 excretion pattern in the food-related atopic dermatitis patients when compared to the normal babies. From this data we concluded that food allergies influence the liver function.

In human study it is very difficult to get a liver sample from a baby. Therefore we conducted the animal study to resolve the relationship between food allergies and the liver.

In the animal experiments, the level of serum ALT in sensitized animals significantly increased in comparison with nonsensitized animals. A hepatic morphology study showed a slight abnormality in the sensitized animals.

These results demonstrate that liver abnormalities appeared in the food-allergy animals.

We treated the sensitized mice with TJ-41 and FOS. Recently it has been suggested that intestinal microflora play an important role in allergic diseases (4, 7). Some studies have reported that probiotics alleviate allergic inflammation (1, 3). When treated with FOS, the intestinal microflora functions improved and favored the production of organic acid such as butyrate, an important metabolite of intestinal bacteria as an anti-inflammatory agent (5).

In the TJ-41 study, we found obvious changes in the villous edema and inflammatory cell filtrations in the small intestines of the control group. On the other hand, the TJ-41 group showed only light inflammatory changes in the small intestines.

When the gastrointestinal tract is influenced, foods that are not well digested still have antigens that can be absorbed by the liver (6).

In our previous experiment, we observed that the number of IgE positive cells and Th2 cytokine expressions increased in the livers of mice with food allergies.

These results suggested that an IgE-mediated response also occurred in the liver of sensitized mice, and the levels of Th2 cytokines increased. However, our data demonstrated that TJ-41 reduces the allergic reaction by lowering the activity of cytokines associated with the immediate allergic response.

From these experiments, we find that dietary FOS may be beneficial for allergy prevention, and TJ-41 is very important in preventing allergic reactions associated with food allergies.

Our results imply that controlling allergic reactions in the small intestine is vital if normal liver function is to be maintained.

### REFERENCES