Effects on the Growth of Mice of Orally Administered Lectin from Taro Tuber (*Colocasia antiquorum*)

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We examined the adverse effects of Taro tuber lectin administered to mice not by stomach intubation but by ad libitum feeding after being mixed into a basal casein diet. The aim of this work was to complete our studies on the in vivo nutritional effects of Taro tuber lectin on the growth of mice, and the effects on the physical activity and energy metabolism of mice which had ingested Taro tuber lectin. Crude and pure lectins were separately ingested for 6-9 days by ddY male mice weighing approximately 15 g, the average daily intakes being 142.1 mg of crude lectin and 71.5 mg of pure lectin. Body weight and food intake were recorded daily, and at the end of the experiment, the internal organs were weighed and the intestines observed under a light microscope. The results elucidated the mechanism by which the toxic effects of this lectin on physiological functions are brought about. The addition of this lectin to a diet resulted in the decreased absorption of dietary nutrients in the small intestine, and the resulting malabsorption induced a reduction in food consumption, which was followed by growth retardation and a tendency toward diminished physical activity and energy metabolism. These results provide substantial evidence that this lectin is responsible, at least in part, for the nutritional inferiority of Taro tuber because of its interference with the intestinal absorption of nutrients.

(Received November 2, 1989)

Keywords: Taro tuber lectin, growth rate, nutrient absorption, small intestine, physical activity, energy metabolism.

INTRODUCTION

Lectins are multivalent carbohydrate-binding proteins of non-immune origin.1) Due to their ability to bind to cell-surface carbohydrates, they have been widely used as tools for exploring the structure of and interaction with cell surfaces.2)3) It has been recently shown that they are involved in immunological events4) and translocation of cancer cells5) when used as such tools, although their physiological functions in the original organisms are not yet well understood. Lectins are present in a variety of foods such as vegetables, fruits, nuts, oils and cereals.6) Especially, they are abundant in the seeds of legumes.7) Since lectins are generally regarded as an antinutritional component of foods, the fact that lectins are so widely distributed in foods commonly consumed by human beings raises the important question of whether they pose any significant risk to human health. Many lectins have been intensively studied with respect to their chemical properties, but relatively little is known about the mechanism for their adverse nutritional effects, the physiological functions of ingested lectins still being unclear. It seems to be difficult at present to assign a unanimous physiological function to lectins from different sources. Therefore, it is necessary to examine various types of lectins from the nutritional viewpoint.

In our laboratory, 27 kinds out of 53 locally available plant foods were found to contain both lectins which agglutinated mouse erythrocyte and toxic substances which caused the death of experi-
mental animals after intraperitoneal injection. Among them, we chose Kintoki bean lectin (KBL) and Taro tuber lectin (TTL) to gain more insight into the possible functions of lectins, especially their physiological effects on mice and rats. The results of the studies on KBL strongly suggested the occurrence of binding of the ingested lectin to epithelial cells of the intestine, leading to a disturbance of the absorption of major nutrients by the intestine and finally to severe growth retardation of the experimental animals. However, bean lectins are easily inactivated when the beans are cooked, thereby leaving no practical toxicity. On the other hand, TTL is relatively resistant to heat treatment and, therefore, may have some toxic effect or antinutritional effects on human beings, if it has physiological properties similar to those of KBL. This was the main reason for undertaking a series of nutritional studies on Taro tuber lectin. In a preliminary experiment, we have already observed some antinutritional effects of TTL administered by stomach intubation on the growth of mice. When mice on a 1% albumin diet were given crude or pure TTL by stomach intubation for 6 days, they showed growth retardation and decreases in food intake, protein efficiency ratio (PER), physical activity and some intestinal enzyme activities (unpublished data).

These results were re-evaluated in the present study to confirm that TTL is a major factor contributing to the in vivo toxicity in mice when mixed into a diet, TTL also being one of the toxic constituents of plant foodstuffs. The present experiments were conducted to make clear whether the lectin isolated from Taro tuber is toxic to mice when ingested ad libitum. In order to carry out this experiment under normal physiological conditions, a 10% casein diet containing crude or pure TTL was given to mice ad libitum, instead of being given by force-feeding with a stomach tube, the results of which will be described elsewhere.

MATERIALS AND METHODS

1. Preparation of the lectins from Taro tuber

Lectin was purified from Taro tuber by means of ion-exchange chromatography on CM-cellulose as previously described, the yield being about 14 mg from 100 g of Taro tuber. Crude lectin was obtained as a precipitate from an aqueous extract with ammonium sulfate. The amounts of the crude and pure lectins required to completely agglutinate 1 ml of a 1% mouse erythrocyte suspension were 62.5 μg and 15.6 μg, respectively, and thereby their specific activities were calculated to be 16 units and 64 units per mg of protein. Hemagglutonins have been generally regarded as being responsible for the growth retardation and death of animals when administered intraperitoneally. Thus, in this experiment, the hemagglutinating activity (HA) and lethal activity (LA) were used as parameters for the toxicity of the crude and pure lectins.

2. Antinutritional effects on mice

Metabolic studies were conducted over a period of 6–9 days on groups of mice fed on a diet with or without TTL. During this period, the growth rate, feed efficiency, absorption of major nutrients, physical activity and energy metabolism were estimated, before a morphological examination of the intestine. Male mice (ddY, from Shizudokyo, Shizuoka) weighing about 10 each g were initially fed on a 10% casein basal stock diet until they attained a weight of about 15 g. The stock diet contained casein (10%), wheat starch (77%), cellulose powder (2%), soybean oil (5%), mineral mixture (5%), and vitamin mixture (1%).

1) Experiment 1

The mice were matched in pairs of similar initial weight, approximately 14–15 g, and divided into one control group and two experimental groups of 6 mice each. They were individually housed in wire-mesh cages in an environmentally controlled room kept at 24 ± 1°C with a 12 hr light-dark cycle. All the mice were given the diet and water ad libitum for 6–9 days. The experimental groups were daily offered the basal stock diet containing either crude or pure lectin, the contents of the lectins being 3.2% and 1.6% of the basal stock diet on a dry weight basis, respectively. The control group was fed only with the basal casein diet. Body weight and food consumption were recorded at 13:00 every day just before replenishing the diets. For 3 days before the end of the experiment, urine and feces were collected to estimate the digestibility of the major nutrients, metabolic cages with a glass separator (MM model, Sugiyamagen Co.) being used for the daily collection of urine and feces. The diets and feces were analyzed to determine the total carbohydrate and
nitrogen in order to calculate the carbohydrate and protein digestibility. Total nitrogen was estimated by the semi-micro Kjeldahl method, and carbohydrate was determined according to the Somogyi-Nelson method. Lipids were not determined because of a shortage of the amount of feces available for analysis.

2) Experiment 2

After 6–9 days of consuming one of the diets, all the mice were fasted for 12 hr and then dissected for a comparison of their tissue weight and a morphological examination of the intestine under a light microscope. Three mice in each group were used in Experiment 3 during the last 2 days to estimate the physical activity and energy metabolism. The fasted mice of each group were killed at the end of the experiment, and the liver, kidney, lung, heart, spleen and small intestine were excised and weighed for each mouse. Fragments of these tissues were fixed for 12 hr in a 4% glutaraldehyde solution buffered at pH 7.3 in 0.05 M phosphate-buffered saline (PBS), embedded into paraffin and sectioned according to the usual histological techniques. The sections were stained with hematoxylin-eosin and observed under a light microscope. Some of the excised small intestine was cut into several sections of 2 cm length. The contents were rinsed out, and the sections were everted, fixed for 10 min in a 4% glutaraldehyde solution, washed with water and then freeze-dried. These everted intestine samples were also observed under a light microscope at a low magnification (×16 or 40).

3) Experiment 3

On the last 2 days of ad libitum feeding, three mice each were selected from the control and two lectin groups to estimate the physical activity (PA) and energy metabolism (EM). The difference of average body weight per capita was 3–4 g between the control and each of the experimental groups. The three mice of each group were individually housed in plastic cages specially designed for the simultaneous estimation of PA and EM, with open circulation of air regulated at a flow rate of 2 l per minute and monitored down to 0.01 l every second by a thermal mass flow sensor. The mice were given ad libitum the same type of basal casein diet with or without TTL as on the preceding days, and measurements were carried out for two successive days. PA was measured by using Animex III, a unit of apparatus for measuring the physical activity of laboratory animals (Shimadzu Co., Kyoto). EM was measured on the basis of an analysis of respiratory gases (O_2 and CO_2) in the outlet air from the animal chambers with a mass spectrometer (WS-MR-1400, Westron Co., Chiba), the accuracy of which enabled us to measure the energy expenditure of small animals. Data for the gas analysis were processed in a micro-computer (NEC, PC-9801 VM21) to calculate the average energy expenditure per minute.

3. Statistical analysis

Student’s “t” test was used to determine significant differences among various experimental values.

RESULTS AND DISCUSSION

Table 1 and Fig. 1 summarize the data for weight changes observed when the mice were fed on the control diet or either of the lectin-containing diets for 6 or 9 days. Difficulties in preparing a sufficient amount of pure lectin led to the shorter experimental period of 6 days, while the experiment with crude lectin was performed for 9 days. Comparisons were made between the control group and each of the lectin groups. Growth retardation, depressed food consumption, and inferior feed efficiency and PER were observed with the mice fed ad libitum on the casein diet to which TTL, regardless of the purity, had been added. The appetite depression due to the presence of TTL in the diet also accounts for the failure of mice to thrive normally when they were fed the lectin-containing diets. Our preliminary experiment indicated that the mice would recover to normal a few days after the lectin had been removed from the diet. Since the absorption of nutrients is a key function of the intestine, the effects of feeding TTL on the absorption of protein and carbohydrate were also examined. In the mice ingesting crude TTL, the absorption rates of protein and carbohydrate were lowered to 95.8% and 96.9% of those of the control group, respectively. The addition of pure TTL to the diet also lowered the absorption rates to 90.4% and 97.3%, respectively, when compared with those of the control mice. In general, both the protein and carbohydrate digestibility was relatively low in the group of mice that had received the casein diet containing
Nitrogen-balance studies have revealed a marked interference with the absorption of nitrogen due to the presence of raw legumes in diets. Larger loss of nitrogen in the feces and, particularly, in the urine of rats fed on raw beans having lectins also seems to point to a promotion of the catabolic breakdown of body tissues. This effect has been attributed to the systemic toxicity caused by lectins which passed across the intestine and penetrated into blood stream, and/or to the systemic effects of bacterial toxins arising from bacterial overgrowth in the small intestine. Although the values for feed intake, feed efficiency and PER of the mice fed on TTL were smaller than those of the control mice, none of the mice died in the experimental groups, which indicates that TTL is relatively less toxic to mice. A dose of around 100 mg per day is large enough to lead to the death of small animals for most bean lectins.

Table 2 shows the tissue weights of the control mice and the experimental mice of the pure TTL group. When TTL was given ad libitum, only the spleen weight was decreased. However, neither an expanded spleen, congestion nor color change in the tissue was observed, which have usually been found in the case of intraperitoneal ingestion. No significant changes in the liver and pancreas weights that often accompany the ingestion of trypsin inhibitor were found in the present experiment. Neither lesions nor enlargement was found histologically under a microscope in thin sections of the liver, spleen, heart, kidney, lung and stomach (pictures not shown). However, in the small intestine of the experimental groups, morphological disorders of the villi-like disarray or ruffles were observed, as well as some alteration to the surface (Fig. 2). In the proximal part of the small intestine of the mice fed on TTL, the villi were irregularly arranged, and they were shorter and less abundant than those of the mice fed only on the control casein diet. Some distur-
bance of digestion and absorption apparently seems to have occurred in the proximal part of the small intestine of the experimental mice where the microvilli are studded. Conceivably, these observations suggest that TTL has potency to cause a malabsorption syndrome. In view of the general ability of lectins to bind to the epithelial cells, although not yet confirmed in the case of TTL, and the resulting decrease in the rates of intestinal absorption/digestion of the main nutrients in mice, the intestine is regarded as the first and main target for the toxicity of TTL fed orally. It can be accepted that lectins in general bind to glycoconjugates on the luminal surface of the gut, causing such morphological changes as those just mentioned or as reported in the literature, and lead to a functional retardation of the intestine.

The physical activity (PA) and energy metab-

Table 2. Comparison of tissue weight* from mice fed on a diet with or without pure lectin

<table>
<thead>
<tr>
<th>Internal organs</th>
<th>10% casein</th>
<th>Pure lectin</th>
<th>% to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1.354±0.018</td>
<td>1.314±0.249</td>
<td>97.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.396±0.029</td>
<td>0.386±0.022</td>
<td>94.5</td>
</tr>
<tr>
<td>Spleen**</td>
<td>0.081±0.002</td>
<td>0.066±0.002</td>
<td>81.5</td>
</tr>
<tr>
<td>Lung</td>
<td>0.143±0.010</td>
<td>0.139±0.007</td>
<td>97.2</td>
</tr>
<tr>
<td>Heart</td>
<td>0.117±0.008</td>
<td>0.115±0.005</td>
<td>98.3</td>
</tr>
</tbody>
</table>

Means±S.D. (n=6).
* Tissue weight is expressed as g per 20 g of body weight. ** Means significantly different from the control group, p<0.05.

Fig. 2. Light micrographs of the jejunum from mice fed on a diet with or without pure Taro tuber lectin
(A) Control mice fed on a 10% casein basal diet (×16)
(B) Experimental mice fed on a diet with lectin (×16)
(C) Control mice fed on a 10% casein basal diet (×40)
(D) Experimental mice fed on a diet with lectin (×40)
The EM of the mice which had ingested a casein diet with or without TTL were continuously measured for two consecutive days with an Animex III and a mass analyzer. Table 3 shows the results of the measurement of the two parameters, PA and EM, which are expressed as average values of the 2-day determination. The total PA and EM in the crude lectin group fell to 79.0% and 81.7%, and those in the pure lectin group leveled to 81.3% and 84.1%, respectively, as compared with those in the control group. Although the number of mice used in this experiment was small because of the time-consuming experiment, and there were statistically significant differences in the parameters between the control and experimental groups, there were none between the crude and pure lectin groups. The decreases in PA and EM seem to be slightly larger than the decreases in the nutrient absorption already described. However, since it has been suggested that lectins can be absorbed intact into the blood stream, an inhibitory effect of the absorbed lectins on erythrocytes, and thereby on the oxygen supply to whole body, may have occurred and account for the present results. In Fig. 3, PA and EM of mice fed on the casein diet with or without pure lectin from Taro tuber are shown at 2 hr intervals as a percentage of the total daily count and caloric value in the control group. In general, movement was more active through the night, namely from 19:00 to 5:00, and during that time period, the energy expenditure was also larger than during the light period. This nocturnal pattern indicates that the change in PA is well compatible with that in EM.

The physiological role of lectins ingested together with foods is a target of interest in the field of nutrition. Teleological questions of this kind can be hardly answered, but experimental evidence should be accumulated in favor of one or another hypothesis. In view of our data on the physiological functions of the lectin purified from Taro tuber, we conclude that the lectin is toxic to mice, although not as strongly toxic as Kintoki bean lectin, and the first target for its oral toxicity is the small intestine. We also assume that this lectin, when ingested, must be stable enough during its passage in the digestive tract to display its toxic activities there. Impairment in the absorption of simple sugars and amino acids by isolated intestinal loops prepared from animals fed with raw legumes or pure lectins points to the occurrence of in vivo interference with absorption of major nutrients. Our present results of the in vivo absorption of carbohydrate and protein also suggest that the capacity of the small intestine to absorb nutrients is generally disturbed by TTL. The decrease in food consumption may also account in part for the antinutritional effects of TTL. Kintoki bean lectin has already been identified in our laboratory as a principal component responsible for the poor nutritive value of the bean. The most obvious manifestation of the toxicity of Kintoki bean lectin was severely impaired growth.

<table>
<thead>
<tr>
<th>Diet</th>
<th>10% casein</th>
<th>Crude lectin</th>
<th>10% casein</th>
<th>Pure lectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>14.0 ± 0.3</td>
<td>14.8 ± 0.5</td>
<td>15.1 ± 0.7</td>
<td>15.6 ± 1.0</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>23.4 ± 1.0</td>
<td>20.8 ± 0.7</td>
<td>22.3 ± 0.6</td>
<td>19.8 ± 0.1</td>
</tr>
<tr>
<td>Lectin intake (mg/day)</td>
<td>0.0</td>
<td>157.5</td>
<td>0.0</td>
<td>70.5</td>
</tr>
<tr>
<td>Physical activity counts/day</td>
<td>2,995 ± 224</td>
<td>2,366 ± 221*</td>
<td>2,894 ± 251</td>
<td>2,352 ± 101*</td>
</tr>
<tr>
<td>% to control</td>
<td>100.0</td>
<td>79.0</td>
<td>100.0</td>
<td>81.3</td>
</tr>
<tr>
<td>Energy expenditure cal/day</td>
<td>15,158 ± 931</td>
<td>12,381 ± 828*</td>
<td>14,254 ± 863</td>
<td>11,992 ± 686*</td>
</tr>
<tr>
<td>% to control</td>
<td>100.0</td>
<td>81.7</td>
<td>100.0</td>
<td>84.1</td>
</tr>
<tr>
<td>Measurement period</td>
<td>last 2 days</td>
<td>last 2 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means ± S.D. (n=3).
* Means significantly from each control group, p < 0.005. Physical activity was measured with an Animex III. Energy expenditure was measured with a mass analyzer.
followed by the eventual death of the experimental mice. The present study indicates that Taro tuber lectin possesses less toxic properties to mice than Kintoki bean lectin. Nevertheless, the histochemical results observed in the mouse small intestine do call for further study on the binding of Taro tuber lectin to the small intestine and on the subsequent functional deterioration of the intestine.

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さといもレクチンのマウスに対する抗栄養作用
—自由摂取時の影響—

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平成元年 11 月 2 日受理

本研究では、さといもレクチンをカゼイン食に混合し自由摂取させることにより、そのマウスに対する抗栄養作用を調べた。この研究の目的は、マウスに対するさといもレクチンの生体での栄養効果の効果を検討し、あわせて運動量やエネルギー代謝に対する影響を調べることであった。約 15 g の雄マウス（ddY）に粗レクチンか純レクチンをnon 9 日間摂取させた。1 日の平均摂取量は、
粗レクチンが 142.1 mg, 純レクチンが 71.5 mg であった。毎日の体重と摂食量を測定し、期間終
了後は臓器重量を測定、小腸の組織像を顕微鏡下で観察した。今回の実験結果からレクチンの毒性
について次のような結論を得た。レクチンを飼料に混合して与えると小腸における栄養素の吸収率が
低下し、次第に摂食量が低下しつつ成長も著しく、それに伴い運動量やエネルギー代謝量も低下す
る傾向がある。

キーワード：さといもレクチン, 成長抑制, 消化吸収率, 反転小腸, 運動量, エネルギー代謝量。