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**Exploratory Trial Concerning Activation of Human Natural Immunity
by Continued Consumption of a Broccoli Extract Processed Food Product**

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ABSTRACT

Objectives: A broccoli extract processed food product has been shown to activate natural immunity in animals, and this study was conducted to examine the natural immunity activating action of continued consumption of this food product in humans and to confirm its safety. **Methods:** Twenty healthy individuals (8 men and 12 women with an average age of 57.5 ± 11.6) consumed 20 mL of a broccoli extract (brolico) every day for 4 weeks. Samples of their blood were collected before and after consumption of the extract, and the activity of NK cells and other cells involved in immunity was measured.

Results: One subject stopped consuming the extract in the middle of trial because it was unpalatable for him. Four subjects could not come to the examination after consumption. Therefore, 16 subjects (6 men and 10 women with an average age of 58.2 ± 10.8) were examined pertaining to the effect of consumption of the extract. It was observed that through continued administration of the trial extract neutrophil phagocytic capacity and NK cell activity in the blood tended to increase. No serious side effects that could be thought of as having been caused by the trial extract were seen during the period of consumption.

Conclusions: The results of this trial suggest that continued consumption of this broccoli extract increases human natural immunity, but randomized clinical trials on a larger number of subjects are now required. (*Jpn Pharmacol Ther* 2012 ; 40 : 489-94)

KEY WORDS: Natural immunity, Natural killer cells, Broccoli, Brolico, Immunostimulator

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Introduction

Living a long, healthy life is a desire common to all humankind, and many people hope to maintain their independence and enjoy a vibrant life in their old age.

Strong immunity is important for realizing a long vibrant life, and immunity is said to be the essence of vital force. If the immune system is robust enough, it can reduce the risk of developing a disease even when infection with a virus or pathogenic bacterium has occurred. Natural killer (NK) cells, known to play a major role in the innate immune system, constantly patrol the body and destroy cancer cells and virus-infected cells.

Many types of food are known to enhance immunity. For example, certain mushrooms, such as shiitake, are known to contain β -1,3-1,6 glucan, a compound that shows anti-tumor and immunopotentiating activities¹⁻⁶); this compound, in its purified form, has been approved as an anti-tumor drug. However, for most health food products claimed in advertising to improve immunity, there is limited evidence to support immunopotentiating effects in humans.

Ishii et al. of the University of Tokyo Graduate School of Pharmaceutical Sciences have found that muscles of silkworm larvae contract by the action of the innate immune system⁷). Imagine Global Care Co., Ltd. has focused on this finding and explored, in collaboration with the University of Tokyo Graduate School of Pharmaceutical Sciences, for immunostimulatory substances using muscle contraction of silkworms as an indicator⁸). As a result, the broccoli extract was found to have the potential for displaying an immunostimulatory effect that may exceed that of β -glucan. Because this effect can be detected only when broccoli constituents are extracted using a unique method⁹), we do not expect normal consumption of broccoli to exert the effect.

Broccoli is a green and yellow vegetable that is native to the Mediterranean coast area and is related to kale, a vegetable known as an ingredient of green juice. Green flower buds and stems of broccoli are edible and rich in vitamin B, vitamin C, carotene, and iron. Unpublished studies of the molecular structure of the extracted broccoli component responsible for the immunostimulatory action by researchers at the University of Tokyo have revealed that it is a novel substance, and it has been named brolico. The effects of sulforaphane, a component in broccoli sprouts, are well known in Europe and the United States¹⁰⁻¹²); however, broccoli sprouts have been found to have less potent natural immunity-activating activity than broccoli in experiments using silkworm muscle contraction as an indicator (unpublished).

In this study, we investigated the activating effect on human immune cells and safety of continuously consumed brolico.

This study was conducted in accordance with the Declaration of Helsinki (1964), Tokyo, Venice, Hong Kong, Somerset West, Edinburgh revision, in reference to the concept of "Ministerial Ordinance on Standards for Clinical Trials of Drugs (new GCP)" (ordinance 28 dated March 27, 1997, Ministry of Health and Welfare), and in accordance with the trial protocol, with approval from the ethical review committee of Imagine Global Care Co., Ltd. Written consent was obtained from subjects after benefits and disadvantages of participating in the study were fully explained.

I Methods

1 Test Food

Broccoli extract (crude) was prepared as the test food. Subjects consumed 20 mL of this extract once daily; 20 mL of the test food contained 500 mg of extract from 14g of broccoli flower buds. The extract was prepared

as described previously⁹⁾ and was confirmed to exert a natural immunity-activating effect in experiments using silkworm muscle contraction as the indicator^{7,8)}. The test food (20 mL) (500 mg of extract) had approximately 10,000 units of activity, where 1 unit was defined as the amount of activity inducing 15% contraction in a silkworm muscle specimen⁸⁾.

2 Subjects

Subjects were recruited by the employees of Imagine Global Care Co., Ltd. Twenty one people who intended to participate in the study underwent pre-consumption tests. One candidate refused to participate after the pre-consumption tests, and a total of 20 subjects (8 males and 12 females; mean age: 57.5 ± 11.6 years) started consumption of the test food. Subject selection and exclusion criteria were as follows.

1) Inclusion criteria

Candidates who met all the following criteria were included.

① A person whose age at the time of consenting was at least 20 years but not more than 74 years.

② A person who received full explanation of the objective and content of the study, was capable of consenting, voluntarily intended to participate in the study with full understanding of the study, and was able to consent in writing to study participation.

2) Exclusion criteria

Candidates who satisfied the above inclusion criteria but met any of the following criteria were excluded.

① A person who showed an allergic reaction to the test food.

② A person who was taking a drug considered to affect the study by the investigators.

③ A person who at present or in the past had a serious disease, such as liver disease, kidney disease, or heart disease.

④ A person who had his/her digestive organ excised.

⑤ A person who was or may become pregnant or lactating during the test period.

⑥ Other persons determined ineligible by the investigators.

Blood or urine tests of the following subjects were missing: one male and one female subject who could not come for the end-of-study tests because of the Great East Japan Earthquake, which occurred during the study period, and an additional male and female subject who did not come for the end-of-study tests for personal reasons.

3 Study methods

1) Study schedule and method of consumption

This was a single-arm study involving only the consumption group. The study schedule is shown in Figure 1.

The subjects consumed a bottle of the test food (20 mL) once daily before or during breakfast for a period of 4 weeks. During the test period, the subjects recorded the drinking status of the test food, changes in physical conditions felt during the study, etc. in a dedicated diary.

The subjects were instructed not to change any lifestyle habits during the study period, such as eating habits and exercise habits.

2) Endpoints

(1) Efficacy endpoints for continuous consumption

① NK cell activity

② Neutrophil phagocytic capacity

③ IL-6 levels

④ Hemogram

(2) Safety endpoints for continuous consumption

① Physiological test: blood pressure

② Blood tests: AST (GOT), ALT (GPT), γ -GTP, creatinine, BUN, uric acid, albumin, TP, general peripheral blood (blood cell test), neutral

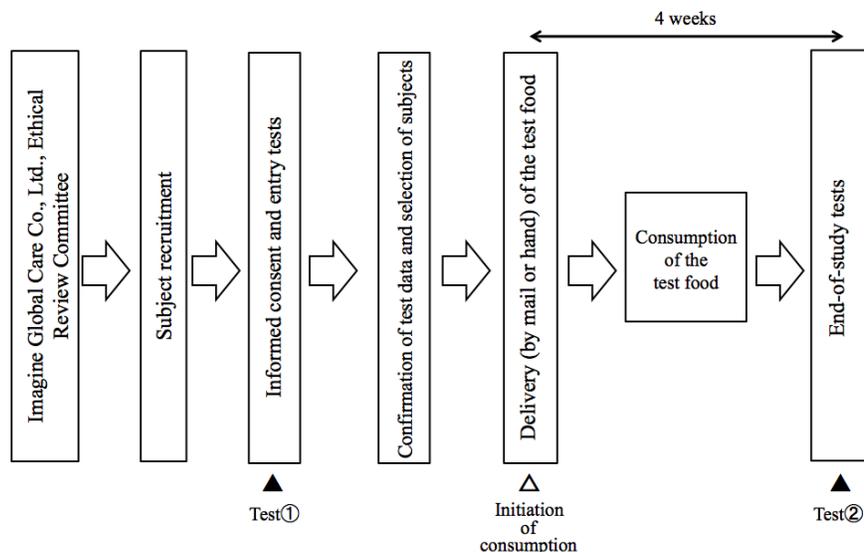


Figure 1: Study Schedule

fat, T-Cho, HDL-Cho, LDL-Cho, fasting blood glucose, HbA1c (NGSP value), Na, Cl, K, Ca, and Mg

③ Urine tests: Occult blood, urine sugar, urine protein, urobilinogen, specific gravity, pH, bilirubin, and ketone body

(3) Subjective symptoms

The investigators checked the subjective symptoms and laboratory test values recorded during the study period and assessed their causal relationships with the test food.

3) Measurement methods

(1) Blood pressure

Blood pressure was measured using an upper arm digital blood pressure monitor (BP-300, Tanita Corp.). The subjects were maintained in a resting state in the sitting position for at least 5 min, and then a nurse measured the blood pressure twice. Of the two measurements, the values from the measurement in which the systolic blood pressure was lower were used for analysis.

(2) Blood collection/urine tests

Blood collection was performed by nurses under supervision of the investigating physicians. Among the blood tests, tests of NK cell activity, neutrophil phagocytic capacity,

hemogram, general peripheral blood, and HbA1c levels were conducted by Mitsubishi Chemical Medience, and other blood tests and urine tests were conducted by SRL Co.

(3) Evaluation of health condition

On both test days, the health condition was evaluated using a self-check form. In case of any unclear description, the investigators or Imagine Global Care personnel directly communicated with the subjects to confirm the absence of any omitted item.

Changes in the health condition during the study period were recorded by each subject in a dedicated diary as they occurred, and adverse events (subjective symptoms) were aggregated on the basis of the records in the diary.

(4) Evaluation of eating habits

On both test days, eating habits over the previous month were recorded using a brief self-administered diet history questionnaire (BDHQ).

4) Statistical analysis

For NK cell activity, neutrophil phagocytic capacity, IL-6 production, other blood tests, and urine tests of the subjects, mean values obtained before and after consumption of the test food were compared by paired *t*-test. Statistical analysis was performed using SPSS 17.0.

Table 1 Effects of brolico consumption on natural immunity-mediating cells

	Before Consumption (mean ± SD)	After Consumption (mean ± SD)
Neutrophil phagocytic capacity (%)	76.1 ± 5.2	77.7 ± 4.6*
NK cell activity (%)	43.6 ± 12.9	50.3 ± 18.6*
IL-6 (pg/mL)	4.1 ± 9.0	2.0 ± 1.8

* p<0.1

Table 3 Comparison of laboratory test values before and after consumption

Test Item	Before		After	
	Mean ±	SD	Mean ±	SD
Diastolic blood pressure (mmHg)	82.75	9.32	77.19	12.18*
Serum glucose (mg/dL)	86.69	19.10	92.13	18.15*
Creatinine (mg/dL)	0.64	0.12	0.67	0.12*
K (mEq/L)	4.08	0.33	4.38	0.38**
Na (mEq/L)	141.75	1.53	140.81	1.60*
Mg (mEq/L)	2.21	0.22	2.06	0.28**
MCV (fL)	94.38	4.49	93.63	4.06**
HbA1c (%)	5.56	0.77	5.69	0.82

n=16

Only items with p<0.1 are shown. *p<0.05, **p<0.01.

II Results

1 Efficacy

1) Changes in natural immunity-mediating cells

NK cell activity and neutrophil phagocytic capacity tended to increase after consumption of the test food (p < 0.1 for both) (Table 1). No significant changes in the hemogram or IL-6 levels were noted between the values before and after consumption.

2) Subjective symptoms

Opinions on the test food based on the diaries recorded by the subjects and heard at the end-of-study tests are summarized in Table 2.

2 Safety

1) Subjective symptoms

Self-reported subjective symptoms included headache (6 subjects), dizziness (1), thirst (1), and constipation tendency (2). Although the attending physicians considered that a causal relationship with the test food could not be ruled out for these symptoms, all symptoms were mild and the subjects recovered within several

Table 2 Subjective symptoms

Subjective Symptom	# of Subjects (out of 16)
Gastritis has improved.	1
Seborrheic eczema has improved.	1
Bleeding of gums has improved.	1
Physical lassitude has improved.	1
Eyestrain has been mitigated.	1
Waking up has become easier.	1
Hay fever has been mitigated.	1
Skin has become shining.	1
Skin texture has become finer.	3

days. No subject discontinued consumption of the test food because of these adverse events.

In addition, the reported symptoms included some cases of sleep deficiency, depression, and stress resulting from the Great East Japan Earthquake that occurred during the study period, but these symptoms were judged as having no causal relationship with the test food.

One subject discontinued consumption of the test food 2 weeks after starting consumption, but this was not due to any adverse event but to personal taste preference.

2) Blood tests/urine tests

The laboratory test values are compiled in Table 3. Diastolic blood pressure was reduced and serum glucose levels were increased. HbA1c levels tended to increase. None of the urine test parameters showed significant differences between the values obtained before and after consumption of the test food.

III Discussion

1 Increasing trend of NK cell activity and neutrophil phagocytic capacity

The observed trend toward increased NK cell activity and neutrophil phagocytic capacity after consumption of the test food suggests that brolico contributes to activation of cells involved in natural immunity. This study was an exploratory study lacking a control group, and the results were somewhat difficult to interpret because of the Great East Japan Earthquake that

occurred during the study period. It is possible that psychological anxiety reduced NK cell activity; therefore, a future study with a larger number of subjects and appropriate control groups is desirable. We are also interested in studying the effects of brolico on the properties of other natural immunity-mediating cells, such as macrophage phagocytic capacity. Animal experiments to elucidate the mechanism of action should also be conducted in parallel to studies in humans.

2 Safety

Although some adverse events for which a causal relationship with the test food could not be ruled out were observed in the present study, all events were mild and did not lead to discontinuation of the test food consumption.

The most frequently reported subjective symptom was headache (6 subjects); however, some subjects had headache as a daily symptom. Although headache was concluded as a symptom for which causal relationship with the test food could not be ruled out in the present study, this conclusion should be confirmed by a larger-scale clinical study in the future.

Among the blood and urine test parameters, serum glucose and Hb1Ac levels were elevated. However, it is unlikely that brolico has an effect of increasing serum glucose levels. Although this parameter is more likely to have been affected by stress from the Great East Japan Earthquake, confirmation in a future study is desirable.

Excluding one subject who discontinued the test food because of personal taste preference after 2 weeks, continued consumption of the test food by the remaining 19 study participants for 4 weeks also supports the safety of brolico.

Conclusion

The objective of this study was to evaluate the effects of a broccoli extract on health, safety, and activity of immune-system cells. NK cell activity, which was the main focus of our study,

showed a tendency to increase upon continued consumption of the test food ($p < 0.1$), suggesting that the extracted broccoli constituent enhances innate immunity. In the future, the immunopotentiating effect of brolico should be tested in further detail in larger-scale clinical studies involving appropriate control groups.

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