

Antihypertensive and Hypocholesterolemic Effects of Tofuyo in Spontaneously Hypertensive Rats

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(Received June 24, 2004; Accepted August 4, 2004;

Published online August 13, 2004)

Tofuyo is a fermented soybean curd that has angiotensin I-converting enzyme (ACE) inhibitory activity *in vitro*. The aim of this study was to examine the antihypertensive and hypocholesterolemic effects of tofuyo *in vivo*. Spontaneously hypertensive male rats at 8 weeks of age were fed a diet containing lyophilized tofuyo for 6 weeks. At 13 weeks of age, the systolic blood pressure of rats in the tofuyo group was significantly lower than that in the control group. After feeding the experimental diets, the ACE activity of kidney was significantly lower in the tofuyo group than that in the control group. Total cholesterol in serum in the tofuyo group was significantly lower while the ratio of high density lipoprotein (HDL) to total cholesterol in the tofuyo group tended to be higher than that in the control group.

Key words — tofuyo, antihypertensive effect, hypocholesterolemic effect, angiotensin I-converting enzyme inhibitor, spontaneously hypertensive rat

INTRODUCTION

Angiotensin I-converting enzyme (ACE, EC 3.4.15.1.) is a dipeptidyl carboxy peptidase that plays an important role in the regulation of blood pressure. It converts angiotensin I to the potent pres-

sor peptide, angiotensin II, and also degrades the depressor peptide bradykinin.^{1,2)} ACE inhibitors in various types of foods have been shown to prevent and alleviate hypertension. The soybean is a valuable source of ACE inhibitors, and some ACE inhibitory peptides have already been isolated from its hydrolysate^{3,4)} and fermented soybean food products.^{5,6)}

Tofuyo is a soybean curd fermented by fungi such as *Monascus* and *Aspergillus* in Okinawa, Japan.⁷⁾ It has a mild flavor similar to cream cheese and has high nutritional value because it is a soybean product. We recently isolated the ACE inhibitory peptides Ile-Phe-Leu and Trp-Leu from tofuyo.⁸⁾ These ACE inhibitory peptides are considered to be liberated from soybean protein during fermentation. ACE inhibitory activities were detected in all fractions separated by column chromatographies during the purification process, indicating that tofuyo should have many ACE inhibitors.

Although ACE inhibitory activity was confirmed in tofuyo *in vitro*, the antihypertensive activity and other physiological functions of tofuyo *in vivo* remain to be clarified. In this study, we investigated the effects of feeding tofuyo on blood pressure, ACE activities in tissues and serum components in the spontaneously hypertensive rat (SHR).

MATERIALS AND METHODS

Materials — Tofuyo was supplied by Benihama Co., Ltd. (Okinawa, Japan). Tofuyo was ground in a mortar and lyophilized. The chemical composition of lyophilized tofuyo powder was analyzed.⁹⁾ As shown in Table 1, tofuyo is especially rich in crude protein.

Experimental Animals and Diets — Male SHR at 4 weeks of age were purchased from Charles River Japan (Shiga, Japan) and fed with a commercial diet for 4 weeks. The rats were housed individually at 25 ± 2°C and 70% humidity under a 12 hr light-dark cycle. At 8 weeks of age, the rats were divided into a control group and a tofuyo group. Each group consisted of 8 rats. Each group was fed a different diet, as shown in Table 2, for 6 weeks. The protein concentration of both experimental diets were 22%. Lyophilized tofuyo (17%, w/w) was added to the diet of the tofuyo group; that is, approximately 5% of total protein was supplied by tofuyo instead of by casein. Sodium chloride at a final concentration of 1% in both diets induced hypertension. The diets

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Table 1. Chemical Composition of Lyophilized Tofuyo

Component	Content (%)
Moisture	17.5
Total protein	30.6
Total fat	21.0
Carbohydrate	26.2
Ash	4.7
NaCl	3.3

Table 2. Composition of Experimental Diets

Contents (%)	Diet group	
	Control	Tofuyo
Casein	25	19
β -Starch	33	33
Sucrose	20.93	16.48
Lard	6.34	6.43
Soybean oil	3.57	0
Mineral mixture	3.74	3.50
Choline chloride	0.15	0.15
Vitamin mixture	1.00	1.00
Cellulose	3.00	3.00
Sodium chloride	1.00	0.44
Lyophilized tofuyo	0	17
Distilled water	2.27	0

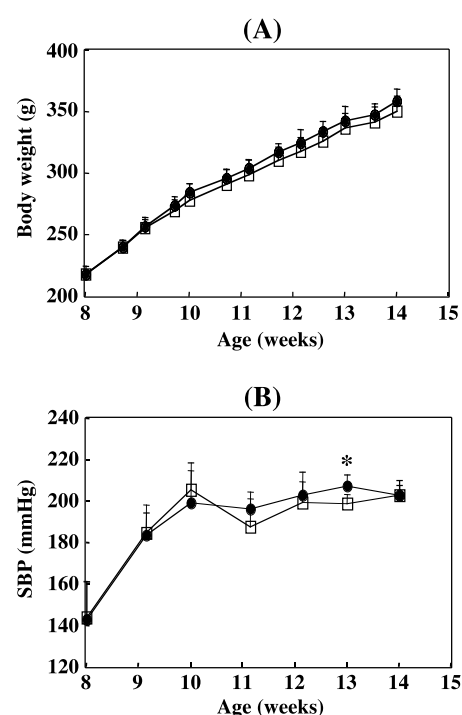
and drinking water were available ad libitum. The experiments were performed under the Guidelines for Animal Experiments at University of the Ryukyus and the Guidelines of the Ministry of Education, Culture, Sports, Science and Technology (No. 141, 1987).

Measurement of Blood Pressure — Systolic blood pressure (SBP) was measured every week by the tail cuff method using a sphygmomanometer (Model MK-1030, Muromachi-Kikai Co., Ltd., Japan) after warming the rat in a warm box kept at 35°C for 10 min.

Analysis of Serum Components and Tissue ACE

At 14 weeks of age, rats were fasted for 16 hr and killed by exsanguination from the heart under pentobarbital anesthesia. Heart, lungs, liver, kidneys and aorta were excised and weighed. Serum of each rat in both groups was collected to measure glucose, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, high density lipoprotein (HDL) cholesterol, triacylglycerol and phospholipid.

ACE extracts of the aorta, lung and kidney were

**Fig. 1.** Changes of Body Weight (A) and Systolic Blood Pressure (B) during Feeding the Experimental Diets in SHR

Each point indicated the mean of eight rats and vertical bars represent the standard deviations. *Asterisk indicates significant difference from control ($p < 0.05$). ●, control group; □, tofuyo group.

prepared by the method of Nakamura *et al.*,¹⁰⁾ and ACE activities were determined by the method of Yamamoto *et al.*¹¹⁾ with some modification. One unit (U) of ACE activity was defined as the amount of enzyme that cleaves 1 μ mol of substrate per min.

Statistical Analysis — Values are expressed as the mean \pm S.D. Student's *t*-test was performed by Statistical Analysis System software (ver. 8.20, SAS Institute, U.S.A.).

RESULTS AND DISCUSSION

Effect of Tofuyo on Growth and Blood Pressure

The growth curves of the rats are shown in Fig. 1(A). The tofuyo group showed a growth rate similar to that of the control group. Diet intake during the experimental period did not differ much between the groups (data not shown).

The SBP of rats gradually increased with body weight [Fig. 1(B)]. At 11 and 12 weeks of age, the SBP of rats in tofuyo group tended to be lower than in the control group, and at 13 weeks of age, the SBP

Table 3. ACE Activities in Serum and Tissues of SHR at 14 weeks of Age

	ACE activity (mU/mg protein)	
	Control	Tofuyo
Serum	1.4 ± 0.1	1.5 ± 0.1
Aorta	155.1 ± 51.0	144.9 ± 45.3
Lung	311.4 ± 132.0	442.9 ± 62.7
Kidney	4.9 ± 1.8	2.6 ± 0.8 ^{a)}

a) Significant difference from the control group ($p < 0.05$).

of the tofuyo group (199.2 ± 4.4 mmHg) was significantly lower than that of the control (207.6 ± 5.4 mmHg). Several studies have examined the antihypertensive effect of ACE inhibitors in SHR by long-term feeding. The SBP of SHR fed sour milk¹⁰⁾ and chicken essence hydrolysate¹²⁾ had decreased 19 mmHg at 23 weeks of age and 26 mmHg at 24 weeks of age, respectively. The experimental period of this study is shorter than those reports, and the decrease of SBP was smaller than those reports.

ACE Activities in Tissues

ACE activities of various tissues are shown in Table 3. The ACE activity of kidney was significantly lower in the tofuyo group than that in the control group. Unger *et al.*¹³⁾ concluded that the persistent ACE inhibition in peripheral tissues, especially vascular wall and kidney, might be important for the prolonged antihypertensive effects of ACE inhibitors such as captopril and enalapril. Although the ACE activities of tissues in this study were measured at 14 weeks of age, that is, after a decline in SBP has been disappeared, the ACE inhibition of kidney in the tofuyo group might be related to the antihypertensive effect of tofuyo.

On the other hand, there were no significant differences in the ACE activities in other tissues including aorta. Nakamura *et al.*¹⁰⁾ and Cheng *et al.*¹²⁾ reported that ACE activity of aorta significantly decreased in SHR fed diets containing ACE inhibitors and the ACE activity of aorta might be important for expression of the antihypertensive effect. Weaker ACE inhibition of aorta in the tofuyo group might be one of the reasons for the weaker antihypertensive effect of tofuyo in this study. In addition, tofuyo may contain not only ACE inhibitors but also other antihypertensive substances, for example, γ -amino butyric acid that exists in red-mold rice which is ingredient of tofuyo and blocks peripheral autonomic ganglia.¹⁴⁾ Further investigations are required to clarify the antihypertensive mechanism of tofuyo and

Table 4. General Composition in Serum of SHR Fed Experimental Diets for 6 weeks

	Control	Tofuyo
Glucose (mg/dl)	114.8 ± 22.9	114.8 ± 15.9
Total protein (g/dl)	6.8 ± 0.3	6.6 ± 0.2
Albumin (g/dl)	2.7 ± 0.1	2.7 ± 0.1
AST (U/L)	212.8 ± 27.0	179.9 ± 31.2 ^{a)}
ALT (U/L)	67.5 ± 10.9	56.3 ± 13.0
Total-cholesterol (mg/dl)	53.8 ± 6.0	46.8 ± 5.0 ^{a)}
HDL-cholesterol (mg/dl)	18.9 ± 1.8	17.0 ± 1.6 ^{a)}
Triacylglycerol (mg/dl)	17.5 ± 11.2	13.6 ± 10.2
Phospholipid (mg/dl)	91.5 ± 11.4	82.6 ± 8.8

a) Significant difference from the control group ($p < 0.05$).

dominant substance for the effect in it.

Serum Analysis

Serum markers are shown in Table 4. There were no significant differences of glucose, total protein, albumin and ALT. The AST in the tofuyo group was significantly lower than that in the control group. However, AST of both groups in this study was higher than that of Wistar Kyoto rat and SHR reported by Fukuda *et al.*,¹⁵⁾ even though some factors such as age of rats and diets in the report were a little different from our study. Although the AST generally increase by the damage of tissue (liver, heart, kidney, *etc.*) or hemolysis, the cause of an increase of AST in this study was unclear on the basis of our present data.

Among the serum lipids, the total cholesterol in the tofuyo group was significantly lower than that in the control group. HDL cholesterol in the tofuyo group was significantly decreased; however, the ratio of HDL to total cholesterol in the tofuyo group tended to be higher than that in the control group. Triacylglycerol and phospholipid in the tofuyo group tended to be lower than that in the control group. These results indicate that tofuyo had hypocholesterolemic activity in serum. In addition, there was no difference in body weight and tissue weights in both groups and no macroscopic liver lesion, such as fatty liver, in either group. Thus, the hypocholesterolemic effect of tofuyo was probably not due to accumulation of lipids in the liver.

Soybeans contain many kinds of hypocholesterolemic constituents, such as saponins, isoflavones, phytic acid and fiber, and soybean protein is considered to be one of the most effective constituents in a diet for cholesterol reduction. Huff *et al.*¹⁶⁾ investigated the hypocholesterolemic effects of soybean

protein isolate (SPI), its enzymatic hydrolysate and amino acid mixture corresponding to SPI in rabbits. Among them, the enzymatic hydrolysate of SPI particularly lowered plasma cholesterol. Iwami *et al.*¹⁷⁾ reported that plasma cholesterol in Wistar rats was significantly decreased and excretion of fecal cholesterol was increased by feeding soybean protein digested by pepsin. Tofuyo is abundant in peptides and amino acids that are liberated from soybean protein during fermentation,¹⁸⁾ which might contribute to the cholesterol reduction in serum. Further studies must be performed using an experimental diet loaded with tofuyo, cholesterol, and cholic acid; lipid concentrations of serum, liver and feces should be analyzed to investigate the metabolism of lipids and the hypocholesterolemic mechanism of tofuyo.

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