

Effects of Casein Hydrolysate on the Systolic Blood Pressure and Serum Lipid Profiles in Spontaneously Hypertensive Rats

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Abstract

The effects of casein hydrolysate on blood pressure (BP) and serum lipid profiles were investigated in spontaneously hypertensive rats (SHR). Twenty-four 6-wk old male SHRs were assigned to 3 groups in a completely randomized design. Experimental groups were as follow: control, market milk group (MLG) and casein hydrolysate group (CHG). Reference blood pressure (RBP) showed average 198.94 ± 1.46 mmHg. Blood pressure (BP) of CHG reduced 24 mmHg after 2 wk of treatment, but these increased after 3 wk. BP of CHG was significantly lower than BP of Control in experimental time ($p < 0.05$). Serum lipid profiles were not differ significantly among groups ($p < 0.05$). These data suggest that casein hydrolysate may beneficially improvement of blood pressure level in SHR.

Key words: casein hydrolysate, blood pressure, serum lipid, spontaneously hypertensive rat

Introduction

Recently, the new relationship between food and health has drawn considerable attention; of interest are the physiological functions of some food components against certain ailments. Among the functions, some foods are known to be effective in suppressing the development of hypertension, which suggests the existence of some components having pharmaceutical action on the regulation systems of blood pressure (Lee *et al.*, 1999). Hypertension is a worldwide problem of epidemic proportions, which presents in 15-20% of all adults. Hypertension is known to induce such direct cardiac circulating diseases as cardiac incompetence, decrease in kidney function, cerebral apoplexy. Hypertension is the most serious and ubiquitous disease and its complications have may contribute to high risk (Do *et al.*, 2007; Wu and Ding, 2001). Food proteins can act as an important source of biologically active peptides with antihypertensive. These peptides are inactive within the sequence of parent proteins, but they can be released by enzymatic proteolysis *in vivo* or *in vitro*. Angiotensin I-converting enzyme (ACE) can convert angiotensin I to angiotensin II which is known to be a strong vasopressor, besides inactivating bradykinin

conducive to lowering blood pressure (Xuan *et al.*, 2005). This enzyme also plays physiological roles in the regulation of local levels of other endogenous peptides, such as enkephalins, neurotensin, and substance P. Therefore, inhibition of ACE can reduce the activity of angiotensinII, but increase bradykinin and enkephalins levels, which results in lowering of blood pressure (Koike *et al.*, 1977). Thus, inhibition of ACE results in a decrease of blood pressure. Peptides derived from Food protein have been well demonstrated having *in vivo* inhibitory activities on ACE and antihypertensive effects after oral administration or intravenous administration in animal experiments using spontaneously hypertensive rats (SHR) and in clinical trials (Li *et al.*, 2004). Many ACE inhibitory peptides have recently been discovered from enzymatic hydrolysates of different food proteins such as garlic (Kim *et al.*, 2005), egg albumen (Kim *et al.*, 2003), buckwheat (Lee *et al.*, 2000), soybean protein (Wu and Ding, 2001), and corn gluten (Suh *et al.*, 2003). Casein shows relatively strong antihypertensive activity against SHR. The ACE inhibiting peptides obtained from trypsin hydrolysates of casein showed the effect of drop of blood pressure in intravenous injection, but there was no effect on oral experiment of trypsin hydrolysates to SHR in the results of Yamamoto *et al.* (1994). Previous studies have shown that the protein hydrolysate had a high ACE inhibitory activity and stable gastrointestinal protease resistance *in vitro*, but their *in vivo* activity needs to be explored at the

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same time (Lee *et al.*, 1999). This study was investigated the hypertensive effect of casein hydrolysate and serum lipid profiles in spontaneously hypertensive rats.

Materials and Methods

Casein hydrolysate

7% Na-caseinate (Virak Co., Food grade, Busan, Korea) solution of distilled water and then each 1% protamex (Novozymes, Bagsvaerd, Denmark) and flavourzyme 500 MG (Novozymes, Bagsvaerd, Denmark) enzyme were added. The mixture hydrolyzed at 55°C for 4 h. The hydrolysis was stopped by heating at 80°C for 5 min (Fig. 1) (Do *et al.*, 2007).

ACE inhibitory activity assay

ACE inhibitory activity was analyzed using a modified version of the method of Cushman and Cheung (1971). The sample (50 µL) and 50 µL Hip-His-Leu (Sigma, St. Louis, MO, USA) were dissolved into 0.1 M sodium

borate buffer (pH 8.3) containing 0.3 M NaCl, and incubated with ACE (50 µL) at 37°C for 30 min. The reaction was stopped by adding 1 N HCl 250 µL. The resulting hippuric acid was extracted by addition of 1.5 mL ethyl acetate. After centrifugation (3000 rpm×5 min), 1 mL of the upper layer was transferred into a glass tube and evaporated at 120°C for 30 min. The hippuric acid was redissolved in 3 mL distilled water, and absorbance was measured at 228 nm using spectrophotometer (V-570, Jasco, Tokyo, Japan). The ACE used in this experiment was extracted from rabbit lung acetone powder (Sigma, St. Louis, MO, USA) 1 g with 0.1 M sodium borate buffer (pH 8.3) containing 0.3 M NaCl 10 mL at 4°C over night and centrifuging for 40 min at 4,000 rpm.

$$\text{ACE inhibition (\%)} = 1 - \left(\frac{Sa - Sb}{Ca - Cb} \right) \times 100$$

Sa: sample absorbance Sb: absorbance of sample blank
Ca: control absorbance Cb: absorbance of control blank

Animals and diets

6 wk-old male spontaneously hypertensive rats (SHR) were used from SLC (SLC, Inc., INASA Branch, Tokyo, Japan). SHRs, a model for genetic hypertension, exhibit higher hypertension than normal rats (Okamoto and Aoki, 1963). All rats were maintained at 18-20°C, 50% humidity, and 12 h on/off light cycle. All of the rats had free access to a standard diet (Table 1) and to drinking water (American Institute of Nutrition, 1977). After acclimation for 1 wk, SHRs were divided into groups of 8 rats. SHRs

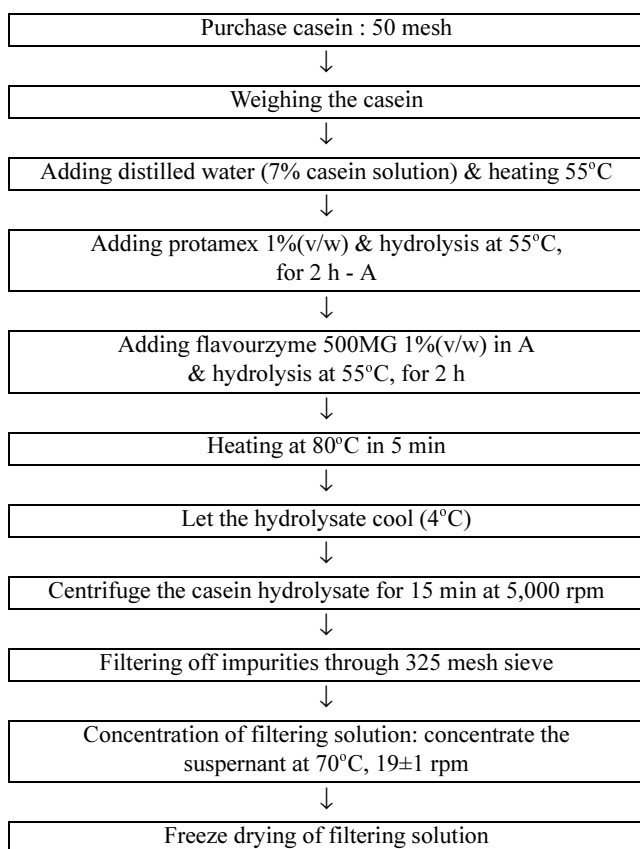


Fig. 1. The production of Casein hydrolysate. 7% casein solution of distilled water and then each 1% protamex and flavourzyme 500MG enzyme were added. The mixture incubated at 55°C for 4 h. The hydrolysis was stopped by heating at 80°C for 5 min. Centrifuge and freeze drying of filtering solution.

Table 1. Composition of basal diet

Ingredients	Contents (%)
Casein (feed grade CP 85%)	20.00
Corn starch	39.75
Dextrinized corn starch	13.20
Sucrose	10.00
Soybean oil	7.00
Cellulose (fiber)	5.00
Mineral mixture ¹⁾	3.50
Vitamin mixture ²⁾	1.00
L-Cystine	0.30
Choline bitartrate	0.25

¹⁾Contained per kg mixture; CaHPO₄ 500 g, NaCl 74 g, K₃C₆O₇·H₂O 220 g, K₂SO₄ 52 g, MgO 24 g, 48% Mn 3.5 g, 17% Fe 6.0 g, 70% Zn 1.6 g, 53% Cu 0.3 g, KIO₃ 0.01 g, CrK(SO₄)₂·12H₂O 0.55 g and sucrose.

²⁾Contained per kg mixture; thiamin-HCl 600 mg, riboflavin 600 mg, pyridoxine-HCl 700mg, nicotinic acid 3 g, Vit.A 400,000 IU (Retinyl acetate), Vit.E(dL-α-tocopheryl acetate) 5,000 IU, Vit. D₃ 2.5 mg, Vit. K 5.0 mg and sucrose.

weighed in the average of 233 g and reference blood pressure (RBP) showed average 223.7 ± 1.2 mmHg.

Experimental diet and conditions

The experimental diets were oral administration and drinking water feeding. The oral administration were randomly divided into 3 groups; Control group (no administration), ML group (oral administration of 2 mL market milk), CH group (oral administration of 10% casein hydrolysate in 2 mL milk). ML and CH groups had oral administration every morning. In drinking water feeding, SHR were randomly divided into 2 groups; Control group, CH group was drinking beverage (1% casein hydrolysate in water). Intake quantity at 2 d once and beverage quantity everyday measured at the time when it is identical.

Blood pressure

The blood pressure of SHRs were assessed by measuring the systolic blood pressure (SBP) every weeks. The blood pressure monitor was used a mercury type sphygmomanometer (IITC Inc., Woodland Hills, CA, USA) in tail vein. RBP was measured before 3 d of casein hydrolysate feeding. Blood classification was assessed according to the standard of the clinical experiment (Han *et al.*, 2003; Pfeffer *et al.*, 1971).

Analysis of serum lipid contents

Blood samples were treated with ethylene diamine tetraacetic acid (EDTA). The collected blood samples were centrifuged for 10 min at 3000 rpm in 4°C, and the supernatant was transferred to an eppendorf tube and stored in a freezer until analysis (Jung *et al.*, 2001; Kim *et al.*, 2007). Serum concentration of total cholesterol (TC) and triglyceride (TG) were measured using kit (cholesterol/triglyceride reagent) of colorimetric assay (ADIVIA 1650, Bayer, Tokyo, Japan). High-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) concentration were measured using kit (direct HDL-cholesterol/cholesterol LDL) of colorimetric assay (ADIVIA 1650/Hitachi 7180, Bayer, Tokyo, Japan) (Rader *et al.*, 2003).

Statistical analysis

All results were expressed in mean \pm SE. Statistical analysis was done using Statistical Analysis System (SAS) program and significance of each group was verified by the Duncan's multiple range test and t-test. Results with $p < 0.05$ were considered statistically significant.

Results and Discussion

ACE inhibitory activity

ACE inhibitory activity of casein hydrolysate was 86.8% (Table 2). Casein hydrolysates by protamex and flavourzyme 500MG at 55°C for 4 h showed promise in the development of a functional food for preventing hypertension. Do *et al.* (2007) have reported casein hydrolysate that casein hydrolysis degrees of flavourzyme 500MG, protamex, and mixture from 1% casein at 55°C for 4 h were 85.5, 88.5, and 93.5%. Yield (93.5%) and ACE inhibitory activity (86.8%) of casein hydrolysate by mixture of protamex and flavourzyme 500MG were the highest. Protamex had the highest activity among endopeptidase activities of hydrolytic proteases showed the same tendency as Kim *et al.* (2002). Furthermore, this result of ACE inhibitory activity was higher than 45.55% in casein hydrolysate by promod 192 (Kim *et al.*, 2002).

Weight gain and food efficiency ratio (FER)

Weight gain and FER during the experimental periods were shown in Table 3. The relative weight gain was higher in control than the other groups, but there was no significant difference in total weight gain (Lee *et al.*, 2000). FER did not show a significant difference among groups. In orally tube feeding, the gain weight of CH group was 60.24 g for 5 wk. The gain weight was higher in control than CH group. FER was control (0.097) and CH group (0.090). Therefore, casein hydrolysate is presumed that food source is used to safety (Park *et al.*, 2000). Ogawa *et al.* (2007) have reported that feeding of osthol was no significant in body weight and daily food intake with control, indicating that dietary osthol gives good growth as well as the control.

Blood pressure

The change in BP after oral administration of casein hydrolysate (2 mL) to SHR for 5 wk is shown in Fig. 2. The reference BP of 6 wk-old SHR was on an average 198.9 mmHg. The systolic blood pressure (SBP) of control during the experimental period was increased from 205 mmHg to 222.4 mmHg. When market milk was

Table 2. Effects of casein hydrolysate on ACE inhibition activity

Enzyme	ACE inhibition (%)
No enzyme	1.5 \pm 0.01
Mixture ¹⁾	86.8 \pm 0.01

¹⁾Casein hydrolysed using protamex and flavourzyme 500MG at 55°C for 4 h.

Table 3. Total gain and FER in SHR fed by orally market milk and 10% casein hydrolysate in milk for 5 wk

Group	Body weight (g)			FER ³⁾
	Initial	Final	Total gain	
Control	234.48±4.38 ^{ns}	300.87±5.43 ^{ns}	66.39±1.05 ^{ns}	0.097±0.024 ^{ns}
Milk ¹⁾	232.63±6.07	292.38±6.55	59.75±0.48	0.098±0.036
CH ²⁾	235.86±3.80	296.19±4.69	60.24±0.89	0.090±0.029

Values are Mean±SD (n=8).

^{ns}Not significant.

¹⁾Milk was orally administered once a day for 5 wk.

²⁾10% Casein hydrolysate in milk

³⁾Food efficiency ratio (body weight/ food intake)

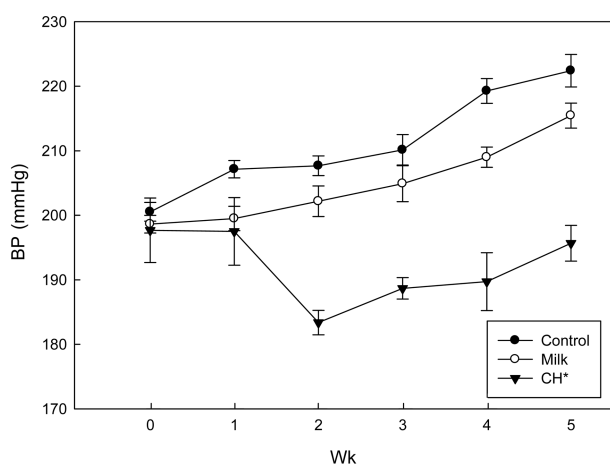


Fig. 2. The changes of systolic blood pressure in SHR fed by orally market milk and 10% casein hydrolysate in milk supplied 2 mL for 5 wk. Blood pressure was decreased by casein hydrolysate for 2 wk. The raising trends of blood pressure were resembled both control and milk groups. Values are the Mean±SD (n=8). BP: blood pressure *: 10% casein hydrolysate in milk.

orally administered to SHR, SBP was increased from 198.6 mmHg to 215.4 mmHg. After oral administration of casein hydrolysate to SHR, SBP was decreased from 197.7 mmHg to 195.7 mmHg for 5 wk. A significant anti-hypotensive effect of casein hydrolysate was observed during the 5-wk experimental period in SHR. BP of SHR is generally increased with weight gain (Lee *et al.*, 2000). The BP during the experimental period was increasing in control and ML groups. But BP of ML group was lower than BP of control group. This result suggests that milk was effective to reduce the blood pressure in SHRs. The SBP of CH group was decreased to 24 mmHg compared to control at 2 wk after oral administration of casein hydrolysate. Generally, the decrease in BP of CH group was significant compared to control and ML groups. On the other hand, the change in BP after drinking water of casein hydrolysate to SHR for 5 wk is shown in Fig. 3. The reference BP of 6 wk-old SHR was on an average

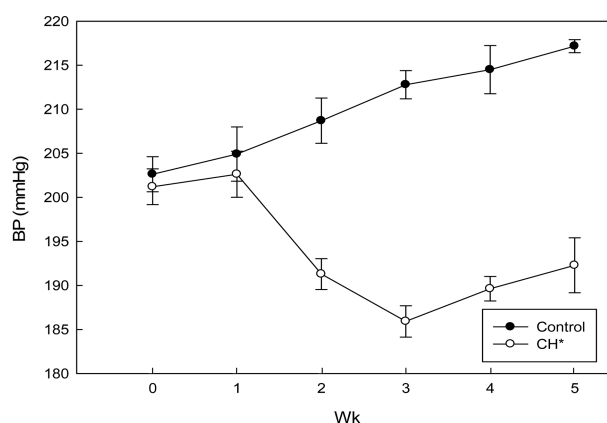


Fig. 3. The changes of systolic blood pressure in SHR fed tap water and 1% casein hydrolysate in water for 5 wk. Blood pressure was decreased by casein hydrolysate for 3 wk. The raising trend of blood pressure was control. Data are the Mean±SD (n=8). BP: blood pressure *: 1% casein hydrolysate in water.

202.0 mmHg. Fig. 3 shows the BP of CH group was significantly lower than the BP of control group at 3 wk after drinking water of casein hydrolysate. The casein hydrolysate in the blood suppressed the activity of angiotensin converting enzymes (ACE) in SHR. Several peptides derived from sour milk, a wheat germ hydrolysate, and a sardine muscle hydrolysate were well known to have ACE-inhibiting activities and to exhibit antihypertensive activities in experimental animals (Do *et al.*, 2006). Ogawa *et al.* (2007) have reported that the increase of systolic blood pressure was significantly suppressed after 3 wk of feeding, indicating that dietary osthol exerted hypotensive activity in SHR. BP of CH group by oral administration was reduced 7.2 % after 2 wk of experimental period. And BP of CH group by drinking was reduced 7.5% after 3 wk of experimental period. BP of CH group was increased after 2 and 3 wk of administration (Fig. 2 and 3). This pattern of reduction in BP, within 2 wk and maintained afterward, was also observed in the Dietary Approach to Stop Hypertension

Table 4. Serum lipid contents and AI in SHR fed by orally market milk and 10% casein hydrolysate in milk for 5 wk

Group	(Unit: mg/dL)									
	TC ¹⁾		TG ²⁾		HDL ³⁾		LDL ⁴⁾		AI ⁵⁾	
	0 wk	5 wk	0 wk	5 wk	0 wk	5 wk	0 wk	5 wk	0 wk	5 wk
Control	65.80± 1.45 ^{aB}	68.40± 1.79 ^{nsA}	99.80± 17.47 ^{nsB}	126.40± 10.04 ^{bA}	25.80± 0.86 ^{aB}	28.00± 0.53 ^{bA}	7.60± 0.63 ^{abNS}	7.20± 0.37 ^{ns}	1.55± 0.10 ^{nsA}	1.44± 0.02 ^{aB}
Milk ⁶⁾	65.00± 3.07 ^{aB}	70.33± 3.99 ^A	93.88± 16.78 ^B	147.33± 14.95 ^{aA}	25.63± 0.88 ^{abB}	29.67± 1.98 ^{aA}	8.25± 0.88 ^{aA}	7.00± 0.58 ^B	1.54± 0.09 ^A	1.37± 0.06 ^{bB}
CH ⁷⁾	61.13± 3.87 ^{bB}	68.43± 3.41 ^A	84.63± 14.65 ^B	132.43±1 9.26 ^{abA}	24.38± 1.69 ^{bB}	29.29± 1.28 ^{abA}	7.00± 0.76 ^{bNS}	6.71± 0.76	1.51± 1.40 ^A	1.34± 0.03 ^{bB}

Values are Mean±SD (n=8).

Same small superscripts are not significantly different by Duncan's multiple range test ($p<0.05$).

Different large superscript is statistically significant by Student t-test for two sampling phase ($p<0.05$).

^{ns} not significant

¹⁾Total-Cholesterol, ²⁾Triglyceride, ³⁾HDL-cholesterol, ⁴⁾LDL-cholesterol, ⁵⁾Atherosclerotic Index = (TC-HDL) ÷ HDL, ⁶⁾Milk was orally administered once a day for 5 wk, ⁷⁾10% Casein in milk

trial (Appel *et al.*, 1997). In that trial, an 8-week intake of a diet rich in fruits, vegetables, and low-fat dairy foods reduced systolic and diastolic BP by 5.5 and 3.0 mmHg more than the control diet in prehypertensive subjects. Similar reductions were observed in our study after 4 wk of repeated administration of casein hydrolysate compared to control group. This shows that casein hydrolysate offers an additional nutritional approach to preventing and treating hypertension, in addition to life style modifications like conscientious dietary choices with reduced salt intake and increased potassium intake, weight loss, exercise, and stress reduction (Appel *et al.*, 1997; Cadée *et al.*, 2007).

Analysis of serum lipids

Serum lipid concentration and atherosclerotic index (AI; Kim *et al.*, 2006) during oral administration of casein hydrolysate (2 mL) to SHR was shown in Table 4. In terms of serum lipids, total-cholesterol (TC) and LDL-cholesterol (LDL-C) concentrations were not significant among experimental groups. Triglyceride (TG) concentration of control was significantly lower than ML and CH groups ($p<0.05$). HDL-C was significantly different between ML (29.67 mg/dL) and control (28.00 mg/dL) ($p<0.05$). AI was lower in ML and CH groups compared to control ($p<0.05$). The comparison between 0 week and 5 wk was observed a significant difference in TC, TG, and HDL-C concentrations by paired t-test ($p<0.05$), except for LDL-C in control and CH groups. However, AI was a significantly decrease after 5 wk ($p<0.05$). These data suggest that casein hydrolysate had a improving effect of LDL-C and AI in SHR. But TG was increase in ML and CH groups. Wu and Ding (2001) have reported that treatment

with soy ACE inhibitory peptides and Captopril administration was no significant changes of content of serum lipids in SHR. Takai *et al.* (2004) have reported that treatment with an ACE inhibitor significantly correlation was observed between SBP and ACE activity in the aorta, but not in the plasma or other tissues such as the heart and brain.

In conclusion, we have demonstrated that casein hydrolysate produced potent antihypertensive effects *in vitro* and *in vivo*. It seems likely that casein hydrolysate would be a beneficial ingredient for a prophylactic and therapeutic treatment against hypertension and its related diseases.

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