Effect of hydrolysate of chicken leg bone protein on attenuating development of cardiovascular hypertrophy in spontaneously hypertensive rats

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Introduction

Hypertension is regarded as a major worldwide health problem and constitutes a high risk factor for development of arteriosclerosis, stroke, coronary heart disease and myocardial infarction. Treatment of hypertension involves the chronic control of blood pressure under normal conditions. It is known that several antihypertensive agents such as Captopril, Lisinopril and Enalapril owe their therapeutic efficacy to angiotensin I converting enzyme (ACE) inhibitory activities. In addition, the ACE inhibitors have been reported to inhibit hypertensive left ventricular hypertrophy more strongly than other first-line antihypertensive agents (Dhlof *et al*., 1992). Currently, many natural ACE inhibitors have been produced by enzymatic hydrolysis of various food proteins and some have demonstrated effectiveness in reducing systolic blood pressure in spontaneously hypertensive rats (SHRs) following their administration. In our previous study, potent ACE inhibitory activity was found in hydrolysate (A4H) obtained from enzymatic hydrolysis of chicken leg bone protein with Alcalase for 4h. The aim of this study was to evaluate the effect of long-term intake of A4H on attenuating development of cardiovascular hypertrophy in SHRs.

Materials and methods

Sample preparation According to the method of Cheng *et al.* (2008), chicken leg bones (broiler) were obtained from a meat processing factory; Tai-Chung, Taiwan. Chicken leg bone was ground with water and heated at 100 ºC for 5min, then digested for 4 h by Alcalase (Sigma, USA) at pH 8.0 and 50°C. The enzymatic hydrolysis was halted by boiling for 10 min and the hydrolysates were centrifuged at 10,000×*g* for 10 min, filtered, lyophilized and stored at -80°C.

Treatment of animals Twenty four male SHRs and eight male WKY rats, seven weeks of age, were raised in an air-conditioned room (25°C) for 1 week, at which point the SHRs were randomly divided into 3 groups and orally administered with hydrolysate (50mg/kg bw), Captopril (1.5mg/kg bw), or water. Tail systolic blood pressure was measured by the tail-cuff method using indirect blood pressure meter (BP-98-A, Softron, Japan). At the end of the experiment (16th week of life), the rats were fasted for 12 h before being sacrificed to weigh their hearts. The hearts of the rats were immediately soaked in neutral buffered 10% formalin, embedded in paraffin, and cut into cross-sections (6 μm thick), which were stained with hematoxylin-eosin and mounted. The thickness of intramyocardial coronary vessel wall was quantified morphometrically using a Leica LB30 microscope (Leica, Göttingen, Germany) with Motic Images Plus2 software (Motic, Xiamen, China).

Statistical analysis Data were analyzed by a GLM program and Duncan's new multiple range test using the SAS System for Windows V8 (SAS, 2000).

Results and discussion

Attenuation of the age-related development of hypertension Figure 1 plots changes of arterial blood pressures of rats with different treatments. The *in vitro* ACE inhibitory activity of A4H was 0.545 mg/mL. SHRs without pharmacological therapy showed elevated blood pressure with increasing age. The lowest blood pressure was observed in WKY rats and remained at about 130 mmHg throughout the experiment. After the second week, significant inhibition was evident in the blood pressure of rats that had been treated with A4H or Captopril ($P<0.05$). At the end of the experiment (16th week of life), the control SHRs had higher blood pressure (211 mmHg) than the A4H-treated (180 mmHg) and Captopril-treated SHRs (178 mmHg). Restated, a significant decrease of about 33 mmHg in blood pressure was finally measured in SHRs that were treated with A4H or Captopril ($P<0.05$).

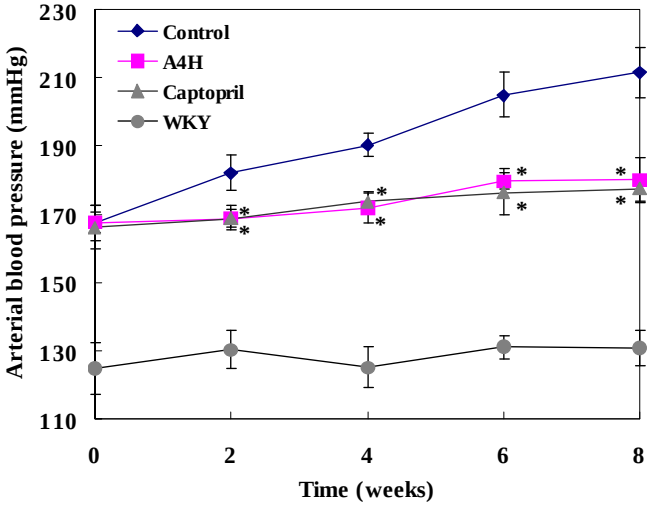

Figure 1. Changes in arterial blood pressure of SHRs treated with four hour incubation hydrolysate by Alcalase (A4H) by oral (50 mg/kg bw/day). Captopril (1.5 mg/kg bw/day) was used as the positive control. WKY and control rats were orally administered with deionized water. *: different significantly from control (P<0.05). n=8.

Attenuation of the development of cardiovascular hypertrophy The heart to body weight ratios of variously treated rats at the end of the experiment (16th week of life) are shown in Figure 2. It was not surprising that normotensive WKY rats had a significantly lower heart to body weight ratio (0.35%) than that of SHRs (P<0.05). In SHRs, the control rats exhibited a significantly higher heart to body weight ratio (0.42%) than the rats treated with A4H or Captopril (0.38%), suggesting that A4H could inhibit cardiac hypertrophy as effectively as Captopril.

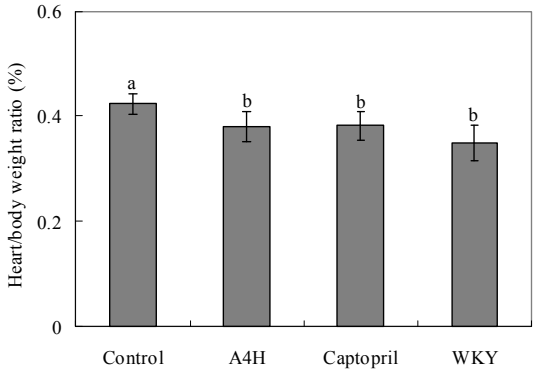

Figure 2. Heart to body weight ratio of rats with different treatment at the end of experiment (16th week). Each value is expressed as mean ± standard deviation. Different superscript letters indicate significant differences (P<0.05). n=8.

Measurements of wall thickness of intramyocardial coronary vessels are presented in Figure 3. Similarly, the wall thickness of coronary vessels in WKY rats (15.8 μm) was significantly lower than SHRs (P<0.05). The wall thickness in control SHRs was 26 μm, which was 2.7 times that of WKY rats. In contrast, SHRs treated with A4H or Captopril had wall thickness of 17 μm and 15 μm, respectively, which were significantly lower than that of control SHRs (P<0.05). Generally, chronic hypertension increases the load on the heart, accelerates the synthesis of myocardium, and causes cardiac hypertrophy (Tsutsui *et al*., 1999). Treatment with ACE inhibitors has been reported to inhibit ACE activity, decrease generation of angiotensin II, reduce arterial blood pressure and attenuate cardiovascular hypertrophy in SHRs. It has also been demonstrated that hypertensive patients can prevent cardiac hypertrophy by maintaining normal blood pressure (Chen *et al*., 1998). Hu *et al*. (2007) reported that the ACE inhibitor Captopril not only had antihypertensive activity but also showed the ability to inhibit cardiac hypertrophy. Moreover, early Captopril treatment of SHR exhibited great therapeutic effects in antihypertension and anti-cardiac hypertrophy (Chen *et al*., 1998). Similar observations were made in this study.

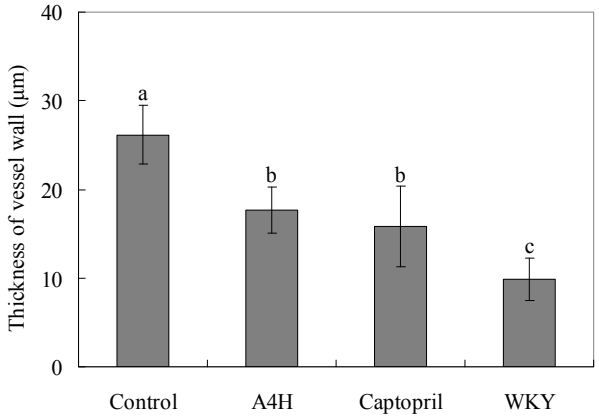

Figure 3. Thickness of coronary vessel walls from rats with different treatment at the end of experiment. Each value is expressed as mean ± standard deviation. Different superscript letters indicate significant differences (P<0.05). n=8.

Conclusions

The observations of this study suggest that A4H with strong ACE inhibitory activity *in vitro* (IC_{50}=0.545 mg/mL) has attenuating effects on the age-related development of hypertension and cardiovascular hypertrophy in SHRs.

References

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