

Original Article

Effect of angiotensin I-converting enzyme inhibitory peptide from rice dregs protein on antihypertensive activity in spontaneously hypertensive rats

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Background: Angiotensin I-converting enzyme (ACE) inhibitory peptide has good effect on blood pressure regulation. Therefore, research to find and develop safer, effective and economical ACE inhibitors is necessary for the prevention and remedy of hypertension. So food-derived ACE inhibitory peptides isolated from food or from enzymatic digestion of food proteins are the safe and efficient substitution for human health.

Objective: To investigate the effect of rice dreg enzymatic hydrolysates as an anti-hypertensive in vivo with spontaneous hypertension rats (SHRs).

Design: The once-oral administration experiments with rice dreg hydrolysates peptide (RDHP) in different doses were conducted using 6 weeks old spontaneous hypertension rats as the test model. Twenty five SHRs were randomized into five groups according to blood-pressure level. The group A is blank control group, the group B positive control group (Captopril), the group C (1.0 mg/kg), the group D (RDHP 10 mg/kg) and group E (RDHP 50 mg/kg). The administration approach is to fill in stomach by mouth. The blood pressure value was observed for each group. The long term oral administration design was conducted for one month. Twenty four SHRs were randomized into 3 groups. The first group is the blank control, the second group is low dose (RDHP 10 mg/kg) and the third group is high dose (RDHP 20 mg/kg). In administration period, blood pressure was measured for once a week.

Outcomes: The once-oral administration animal experiments showed that the blood pressure of SHR drop was 11 mmHg, 17 mmHg, 26.00 mmHg, 17 mmHg at a dosage of 1 mg/kg, 10 mg/kg, 50 mg/kg hydrolysates and 1 mg/kg captopril, respectively, after 1h administration. The results of long-term oral administration indicated that the blood pressure drop was 17 mmHg, 26 mmHg in 10 mg/kg and 20 mg/kg, respectively after 30 days administration. RDHP not only promoted the growth of SHR, but also had no adverse effect on heart rate.

Conclusions: The present study indicated the inhibitory peptides from rice dreg hydrolysate had significant anti-hypertensive action and no other side effects by oral administration in SHR.

Key Words: rice dreg protein, ACE inhibitor, antihypertension, spontaneously hypertensive rats

Introduction

Angiotensin I-converting enzyme (ACE, dipeptidyl carboxypeptidase I, kinase II, EC. 3.4.15.1) is a multifunctional, zinc-containing enzyme, located in different tissues. This enzyme plays a key physiological role in the control of blood pressure, by virtue of the rennin-angiotensin system.¹⁻² ACE converts the inactive decapeptide, angiotensin I, to the potent vasopressor octapeptide, angiotensin II, and inactivates bradykinin.³ The blood pressure regulation of ACE is performed mainly through two means: the one way is to transform angiotensin I into angiotensin II, the other way is through kinin degradation so to make it loss activity.⁴ The antihypertensive function of ACE inhibitor is carried out through the inhibition of the activity of ACE. Thus, inhibition of ACE is considered to be a useful therapeutic approach in the treatment of hypertension. Therefore, in the development of drugs to control high blood pressure, ACE inhibition had become an important target. A large number

of highly potent and specific ACE inhibitors have been developed as orally active drugs that used in the treatment of hypertension and congestive heart failure.⁵

Several ACE inhibitors show antihypertensive effects and may also have beneficial effects on glucose and lipid metabolism,⁶⁻⁸ on decreasing insulin requirements in diabetes, in increasing exercise tolerance, as well as other beneficial effects.^{8,9} Since the original discovery of ACE inhibitors in snake venom, captopril, enalapril and lisinopril, an effective oral inhibitor, have been developed and all are currently used as clinical antihypertensive drugs. Although synthetic ACE inhibitors had good effect on antihypertensive, they can cause adverse side effects.

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Therefore, efforts to develop safer, effective and economical ACE inhibitors are necessary for the prevention and remedy of hypertension. For many years, food researchers have extensively studied peptides derived from food proteins as potential nutraceuticals in respect to the development of functional foods. Many research groups have combed for novel ACE inhibitors in natural products and in microbial sources.¹⁰ Recently, a new relationship between food and health has drawn considerable attention, that being physiological functions of some food components against certain ailments. Bioactive peptides can be released by enzymatic proteolysis of food proteins and may act as potential physiological modulators of metabolism during intestinal digestion of the diet. The possible regulatory effects of peptides relate to nutrient uptake, immune defense, opioid and antihypertensive activities. Up to now, many food-derived ACE inhibitory peptides have been isolated from food or from enzymatic digestion of food proteins,¹¹⁻¹² including gelatin,¹³ casein,¹⁴ fish,¹⁵ fig tree latex,¹⁶ and α -zein.¹⁷ Other ACE inhibitors were isolated from sake and its by-products,^[18] Korean traditional rice wines and liquors,¹⁹ cereals and legumes,²⁰ and microbes such as yeasts.²¹ These peptides are less potent than synthetic ones, but, they do not exhibit known side effects. Nevertheless, there was no report on the ACE inhibition activity and antihypertensive action in vivo model from rice dregs protein.

The in vitro ACE inhibitory activity of bioactive peptide was optimized by enzymatic hydrolysate of rice dregs protein in our previous study.²² In order to further prove the antihypertension activity of peptides by enzymatic hydrolysates of rice dregs, the experimental design in vivo with once-oral administration and long-term oral administration were conducted in SHR. The objective was to investigate and verify the antihypertension activity of purified peptides from rice dregs protein in SHR.

Materials and methods

Materials

Rice dreg proteins were purchased from Sauce Brewery factory of Zhejiang MiFeng Group (moisture 78%). ACE (EC 3.4.15.1) from rabbit lung, hippuryl-histidyl-leucine (HHL) as a substrate peptide of ACE, α -chymotrypsin (EC 3.4.21.1) from bovine pancreas, Sephadex G-15 purchased from Sigma Chemical Co., (St. Louis, MO). Captopril, a commercial antihypertensive drug, was purchased from Kasei Kogyo Co. (Japan). Pepsin, trypsin, trifluoroacetic acid, and acetonitrile were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Unless otherwise specified, all chemicals and solvents were of analytical grade. Male, spontaneously hypertensive rats (SHR) were purchased from the Chinese Academy Sciences. Each of the rats weighs 200g and each was 6 weeks old.

Experimental methods

Preparation of Rice dreg hydrolysate peptide (RDHP): The rice dregs were hydrolyzed by trypsin. The hydrolysis process was terminated by heating in boiling water for 10 min, and the hydrolysate was centrifuged at 2500×g for 20 min to obtain the supernatant. After concentration, the hydrolysates were freeze-dried. Optimum conditions for rice dregs hydrolysis are pH of 8.02, temperature of 37°C,

enzyme to substrate ratio (E/S) of 1.5%, the ratio of water to rice dregs (W/R) 4.13, and hydrolysis time span of 4.0 h. the ACE inhibitory peptides prepared from rice dregs with enzymatic proteinases (the hydrolysis condition seen as ref. 22). The enzymatic peptides (RDHP) were purified with Sephadex G-15 column and collected for further animal experiments according to reference.²³

Feed of spontaneous hypertension rats (SHR): SHR were raised in an experimental animal feeding house with 24°C and 12 hours sunlight per day, take food and water freely. Feeding condition of SHR was temperature 25°C, humidity 70%. The ACE inhibitory peptide purified from RDHP was dissolved in physiological saline at a dose of 10 mg/kg body weight and injected orally using a metal gastric zoned in SHR. The lowering efficacy of peptide on systolic blood pressure (SBP) was compared with that of captopril. Captopril was injected as the same method of the peptide from RDPH. Control rats were administrated with the same volume of saline solution. Following oral administration of sample, SBP was measured by tail-cuff method with a Softron BP system (Softron BP-98A, Tokyo, Japan) after warming SHR in a chamber maintained at 38°C for 5 min. 5 times at a time and take the average. Preparation of RDHP for oral administration: The design was constructed according to experimental dose, weigh ACE inhibitory peptide of rice dregs protein exactly, packed and put in refrigerator at -20°C after dissolved by 0.9% physiological brine, thawed when use.

The once-oral administration experiments of RDHP: To divide 25 SHRs into 5 groups at random according to blood-pressure level, 5 rats for each group, named group A, group B, group C, group D and group E. The group A is blank control group, the group B is positive control group, the group C is low dose group and group E is high dose group. Physiological brine administration for group A, captopril administration for group B, low dose administration ACE inhibitory peptide of RDHP for group C, middle dose administration for group D and high dose administration for group E. The dose for each group in order is 0 mg/kg, 1.0 mg/kg (captopril), 1.0 mg/kg, 10 mg/kg, 50.0 mg/kg (listed in Table 1). The administration approach is fill in stomach by mouth. After administration based on experimental design, the blood pressure value is observed and measured for each group. The time interval for measurement is 0 h, 1 h, 3 h, 5 h, 7 h.

The long term administration experiments of RDHP: To divide 24 SHRs into 3 groups at random, 8 rats for each group. The first group is blank control group, the

Table 1. The experimental design using the once-oral administration doses with SHR

Dose (mg/kg)	SHR number
0.0	5
1.0 (Captopril)	5
1.0	5
10.0	5
50.0	5

Table 2. the long-term experimental design with using different doses with SHR

Dose(mg/kg)	SHR number
Control	8
10	8
20	8

second group is low dose group and the third group is high dose group. Physiological brine administration for blank control group, RDHP administration for middle dose and high dose group, the dose for each group in order is: 0 mg/kg, 10.0 mg/kg, 20.0 mg/kg (in Table 2). The administration approach is filled in stomach by mouth. The administration was one time for each day during one month. In administration period, the blood pressure of rats was measured once a week.

Statistical analysis: All results were expressed as means \pm SEM (n = 3). The significance of the differences between SBPs before and after administration was analysed using Student's t-test. SAS 8.0 software was used in the statistical analysis.

Results

Effect of the once-oral administration of ACE inhibitory peptide from RDHP on blood pressure regulation in SHR

As shown in Table 3, the maximal SBP reduction recorded of 28 mmHg at 1 h after administration of 50 mg/kg body weight was observed. As compared with the control and the captopril (1mg/kg), the administration effect of 10 mg/kg body weight for RDHP peptide was equivalent to that of the 1 mg/kg body weight for capto-

pril product.

Based on the result of Table 3, the blood pressure of SHR for every group began to decline after once-oral administration by ACE inhibitory peptide of rice dregs protein and captopril, the blood pressure drop most evidently at 1h and blood pressure value begin to return after that, the value recovered basically to the level before the administration by 7h. Among these, the blood pressure decline with ACE inhibitory peptide at a dosage of 50 mg/kg is the most evident, the decline extent reached 26.00 mmHg. Among the experimented dosages, the blood pressure decline extent of captopril at a dosage of 1 mg/kg was equivalent to that of ACE inhibitory peptide of rice dregs protein at a dosage of 10 mg/kg. This suggest that the antihypertensive activity of ACE inhibitory peptide form rice dregs hydrolysates at a dosage of 10 mg/kg per rat is possible equivalent to that of captopril at a dosage of 1 mg/kg per rat. It is also obvious in the experiment that the intimate relationship is observed between hypertension activity and dose of ACE inhibitory peptide, it is dose-effect relationship. The statistical results indicated that every experimental group is significantly different from the control group at the level of 99 percentages in 1h, 3h and 5h except for ACE inhibitory peptide of rice dregs protein at a dosage of 1 mg/kg in 5h.

Effect of long term administration experiments of RDPH ACE inhibitory peptide on blood pressure in SHR

Based on the Table 4 results, as rice dregs zymolyte ACE inhibitory peptide was administrated, the decline of SHR' blood pressure at a dose of 10 mg/kg and 20 mg/kg groups was more evident than that of the control along with all the administration time. The blood pressure of two groups at the end of administration time is 181 \pm 3.069 mmHg and 173 \pm 3.251 mmHg, respectively. The drop of

Table 3. The change of blood pressure under once-oral administration of rice dregs protein ACE inhibitory peptide

Dose (mg/kg)	Blood pressure (mmHg \pm SE)				
	0 h	1 h	3 h	5 h	7 h
Control	199 \pm 2.133	197 \pm 2.486	199 \pm 2.115	196 \pm 2.408	198 \pm 2.215
1 *(C)	199 \pm 2.759	181 \pm 2.802**	182.9 \pm 2.949**	190 \pm 2.283**	193 \pm 2.724
1	199 \pm 2.436	189 \pm 2.965**	191 \pm 2.428**	192 \pm 2.793	196 \pm 2.264
10	199 \pm 2.557	180 \pm 3.255**	185 \pm 2.348**	191 \pm 2.139**	195 \pm 2.685
50	199 \pm 2.607	171 \pm 2.608**	174 \pm 2.771**	185 \pm 2.873**	193 \pm 2.425

C: Captopril, ** $p < 0.01$, Values are means \pm S.E. of three determinant.

Table 4. the Change of blood pressure under long term administration experiments of rice dregs hydrolysate ACE inhibitory peptide

Dose (mg/kg)	Blood pressure (mmHg \pm SE)				
	0 week	1 week	2 weeks	3 weeks	4 weeks
Control	198 \pm 2.949	199 \pm 3.236	198 \pm 3.118	199 \pm 3.213	200 \pm 3.439
10	198 \pm 3.328	191.0 \pm 3.582**	186 \pm 2.989**	1820 \pm 3.318**	181 \pm 3.069**
20	199 \pm 3.268	186 \pm 3.138**	181 \pm 3.326**	176 \pm 3.554**	173 \pm 3.251**

** $p < 0.01$, Values are means \pm S.E. of three determinant.

Table 5. The influence of long term oral administration with rice dregs protein hydrolysate ACE inhibitory peptide on SHR's weight

Group	Control	10 mg/kg	20 mg/kg
Body weight (g±SE)	19.33±2.9155	28.00±3.5456**	29.875±3.3568**

** $p < 0.01$, Values are means ± S.E. of three determinant.

blood pressure was 17 mmHg and 26 mmHg, respectively. The statistical analysis results demonstrated that the SHR's blood pressure drop for 10 mg/kg and 20 mg/kg groups administrated with RDHP are significant at the probability of 99% level when compared with that of the control. It suggest that the long term administration of ACE inhibitory peptide from RDPH had beneficial effect on blood pressure regulation of SHR. It was also evident that the dynamic change of SHR's blood pressure was keeping drop with the long term administration for experimental groups. Thus, it supposed that the long-term administration of RDHP was useful for blood pressure regulation of SHR.

The influence of long-term oral administration with ACE inhibitory peptide from rice dregs protein hydrolysate on SHR's weight

It was observed that the SHR's weight was changed under long-term oral administration with ACE inhibitory peptide of rice dregs hydrolysate (Table 5). The experimental result showed that in experimental period, the SHR's weight of the control group increased 19.33±2.9155 g, but the SHR's weight of 10 mg/kg and 20 mg/kg groups increased 28.00±3.5456 g and 29.875±3.3568 g, respectively. The ACE inhibitory peptide from rice dregs protein not only has antihypertension activity but also nourishment function of accelerating growth (Table 5). Furthermore, it was observed the experimental groups of once-oral and long-term administration of ACE inhibitory peptide had no positive intervention on SHR's heart rate (results not shown). The safety of ACE inhibitory peptides was firstly taken into consideration for its successful application in human health.

Discussion

Hypertension is a major risk factor for cardiovascular disease such as heart failure, stroke, coronary heart disease, and myocardial infarction.²⁴ Hypertension is called the "silent killer" for good reason: almost one third of individuals with hypertension do not know that they have it. More amazing is that almost 50% of those who do know they have hypertension do not have it under control. Hypertension is the primary or contributing cause of >200,000. There are a great number of pharmaceuticals that have been proved to be effective in lowering blood pressure. Diet and lifestyle modification may also represent effective tools for the prevention of hypertension, which could decrease the requirement for antihypertensive drugs that usually have side effects during treatment of the disease. In this respect, the search for diet-related preventive measures for hypertension is obviously of interest within the scope of functional foods. ACE inhibitory peptides derived food proteins are just the ideal candidates for such products. Although the ACE inhibition

potencies of these peptides are not as great as those of drugs commonly used in the treatment of hypertension, they are naturally derived from food protein sources, and considered to be milder and safer without the side effects as compared with the drugs. At the same time, these peptides usually have multifunctional properties and are easily absorbed. Therefore, food protein-derived ACE inhibitory peptides show great promise in the development of a novel physiologically functional food for preventing hypertension as well as for therapeutic purposes.

It was well known that almost all ACE inhibitors were peptides, except Ganaderma lucidum, which was a triterpene.²⁷ There were many reported sequences of ACE inhibitors in the range of tripeptide to oligopeptides. Among them, the food-derived peptides had received much more attention. In this study, it was found that the effect of administration with 10 mg/kg body weight on blood pressure after filling 1 h was the same as that of the captopril. Lee et al.²⁵ found that ACE inhibitor from *T. giganteum* showed a clear antihypertensive effect in SHR, at a dosage of 1mg/kg. After 2 h of administrating the ACE inhibitor at a dosage of 1 mg/kg, blood pressure decreased to 157 mmHg, slightly increasing later to the average blood. The drop of blood pressure was 36 mmHg after administration at 2 h. Fujita and Yoshikawa²⁶ reported that LKPNM is a prodrug type of ACE inhibitory peptide because LKPNM was found to be hydrolysed by ACE to produce LKP, which had 8-fold higher ACE inhibitory activity compared to LKPNM. Although hydrolysate peptides of rice dregs protein had no great significant effects as the captopril group on blood pressure drop under the equal administration dose, the rice dreg protein hydrolysate still exhibited the prospected activity and function when compared with those of fish protein peptide and other plant derived ACE inhibitory peptides. The inhibitory action of RDHP on ACE in vitro was the main reaction mechanism, but there are still unknown whether it existed other mechanisms for RDPH against hypertension in SHR. Korhonen and Pihlanto²⁸ reviewed that apart from ACE inhibition, milk peptides may exert antihypertensive effects through other mechanisms, such as inhibition of the release of endothelin-1 by endothelial cells, stimulation of bradykinin activity, enhancement of endothelium-derived nitric oxide production and enhancement of the vasodilatory action of binding to opiate receptors.

In addition to reaction mechanism, the safety of antihypertension product was the most important aspect for isolating the ACE inhibitory peptides from natural resources. The preliminary results of this study indicated that the long-term use of rice dreg protein hydrolysate peptide had no side effects on heart rate and had positive impact on body weight. However, efforts are still needed to keep much more investigations on the non-toxic effects through the Ames test and the MTT assay, its stability

under intestinal proteases and its antihypertensive effect without any adverse side effects, *in vivo*. Furthermore, most important and interesting, molecular studies are needed to assess the mechanisms by which bioactive peptides exert their activities. For this approach, it is necessary to employ proteomics and associated technologies.²⁹ Thus, the sequence and structure of the purified peptide are needed to be determined for providing much more information in the future research. These studies suggest that ACE inhibitory peptide derived from rice dregs protein could be utilized to develop nutraceuticals and pharmaceuticals. In addition, it is expected that this will contribute developing interest in basic research and potential applications of bioactive peptides.

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