Facilitating Production of Rice Dreg Peptides via Lactic Acid Bacterial Fermentation on Anti-Hypertension Efficacy

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ABSTRACT

Rice (Oryza sativa L.), a second major crop production in the world, is a staple food for more than 100 countries. Rice Dreg Peptides (RDP), which is the cheap by-product of rice syrup, contains more than 50% protein content and is used as a protein source. The natural food sources and their by-products have also been reported to possessed numerous regulation of physiologic and biologic functions. At present, a number of chemicals have been effectively used for anti-hypertension. However, there are numerous side effects associated with them. Therefore, the aim of this study was the investigation of the anti-hypertensive effect of RDP via lactic acid bacterial fermentation by using in vitro Angiotensin I-Converting Enzyme (ACE) inhibitory assay and in vivo animal experiment with Spontaneous Hypertension Rats (SHRs). In vitro results were shown that the most ideal fermentation conditions: fermentation time, pH value, and bacterial concentrations are 48 h, 5.17, and 3.6 × 10^10 CFU/mL, respectively.

According to the most ideal fermentation conditions, the concentration of RDP (mg/mL) under three fermentation time were respectively calculated: 24.14 ± 0.09 (fermentation at 0 h), 31.79 ± 0.42 (fermentation at 24 h), and 30.62 ± 0.26 (fermentation at 48 h). In addition, the anti-hypertensive effect of RDP by using in vitro ACE inhibitory assay was presented that 79.34 ± 4.61% at 0 h-fermentation, 84.78 ± 7.72% at 24 h-fermentation, and 96.70 ± 5.85% at 48 h-fermentation. On the other hand, the once per day-oral administration (p.o.) experiments with RDP in two doses were conducted using 6-8 weeks old SHRs. Thirty-five SHRs were randomized into 4 groups [negative control group: 5 rats; positive control group (20 mg/kg/day nifedipine administration): 10 rats; RDP_low group (50 mg/kg RDP): 10 rats; RDP_high group (200 mg/kg RDP): 10 rats] according to blood-pressure level. Ten Wistar Kyoto (WKY) rats were blank normal control group. RDG was administrated by p.o. through gavage per day for 2 months. The blood pressure values were measured for each group once a week.

In vivo results were presented that the blood pressure of SHRs before and after experiment in four groups were respectively 181.20 ± 6.69 mmHg; 179.43 ± 6.90 mmHg; 129.44 ± 3.80 mmHg (positive control group), 171.40 ± 5.29 mmHg; 167.00 ± 0.92 mmHg (RDP_low group), and 169.70 ± 6.69 mmHg; 159.70 ± 1.01 mmHg (RDP_high group). The mean blood pressure in two RDP groups was significantly lower than negative control group. Moreover, 4 indexes of the liver and kidney functions of RDP-administrated SHRs were located at the normal levels. In conclusions, the present study indicated the RDP had significant antihypertensive efficacy and no side effects were found in vitro and in vivo.
Introduction

Rice (*Oryza sativa* L.) represents one of the leading food crops in the world. A global annual production was about 480 million metric tons (milled rice basis) in 2015 [1]. It is cultivated today in more than 100 countries except Antarctica. It is the staple food for over half the world’s population, mainly in Asian countries, where it provides a considerable portion of the protein intake for millions of people [2-3]. The total food protein production of rice per hectare is second only to that of wheat, although the yield of utilisable protein is actually higher for rice than for wheat, due to the superior quality of rice proteins [3-4]. Rice is known to have nutritional, hypoallergenic and healthy properties, which are retained by Rice Dreg Peptides (RDP) derived from rice starch by-products that contains more than 50% protein content and is used as a protein source [5]. With the massive of rice starch syrups industry in China, RDP is available in large amounts at minimal costs. RDP are currently used as animal feed with low economic benefits in China. In China, the development of efficient methods to recovering RDP for human consumption that could significantly increase economic and social benefits [6].

High blood pressure has now become a major global health concern. The disease affected approximately 73 million people in USA [7]. There will be about 1.56 billion people suffered with hypertension worldwide in 2025 [7-8]. At present, hundreds of peptides with antihypertensive activity have already been reported and novel peptides are also discovered every day [7]. These bio-functional peptides were derived from 35 major sources that include animal matrix as milk, egg, fish, pork, and chicken and plant as soybean etc. [7]. In general, plant-derived functional peptides are considered more safe and healthy compounds. They are low molecular weight, possess different biological activities, and are easily absorbed by the small intestine in human [9-10]. Usually, plant-derived functional peptides may show not only antioxidant properties but also a wide range of other physiologic and biological activities as anti-hypertension, anti-hypercholesterolemia, immunomodulation, and anti-microbial actions [5,9-10].

Angiotensin Converting Enzyme (ACE) increases blood pressure by converting the inactive angiotensin I (decapeptide) to the active angiotensin II (octapeptide). Several chemicals have been verified to act as anti-hypertensive drugs. These clinical anti-hypertensive drugs included as nifedipine, captopril, fosinopril, lisinopril etc., which act either by direct inhibition of ACE or block of the angiotensin II receptors. Although these chemicals are effective in the blood pressure control, however, there are many side effects found [11-12]. For the example, in the nifedipine intake, there are many side effects found as mild dizziness, flushing (warmth, redness, or tingly feeling), weakness, headache, mood changes, heartburn, nausea, tremors, muscle cramps, cough, wheezing, sore throat, stuffy nose, taste disturbance, skin rashes, kidney failure etc [7].

Besides chemical drugs, diet and lifestyle also play a significant role in the prevention of hypertension. Lots of small molecular peptides with antihypertensive activity have been discovered and many of these peptides are part of proteins present in our daily food. They are not toxic in general and may represent a new therapeutic strategy for the prevention and treatment of hypertension [7]. Hence, RDP were obtained from the rice dreg fermentation with Pediococcus pentosaceus L11 in this study. This novel RDP with anti-hypertensive activity was verified via in vitro ACE inhibition assay and in vivo SHRs animal experiment. There is a substantial interest in discovering RDP with antihypertensive activity in this study.

Materials and Methods

Food by Products, Chemicals and Reagents

Rice dreg (moisture: 80%) was provided from rice winery in Miaoli, Taiwan. *Pediococcus pentosaceus* L11 (L11) was isolated from soil in Miaoli, Taiwan. Angiotensin-I-converting enzyme (ACE) and Hipurry-L-histidyl-L-leucine (HHL) were ordered from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals used were of analytical grades.

Fermentation of Rice Dreg

Rice dreg was adjusted to pH 7.0 using sodium hydroxide solution and fermented with 5% sucrose and 10% of L11 (final concentration of approximately 10^5 CFU/mL) and inoculated at 37°C for 48 h. Later, these fermented samples were collected at post-fermentation 0, 24, and 48 h, respectively. Then, the fermented rice dreg was centrifuged at 6,000 × g for 30 min and removed insoluble materials. Collection of the supernatant was filtered (Advantec Grade No. 2 Qualitative Filter Paper) then stored at -30°C until analysis.

Measurement of RDP content

Measurement of the peptide content of the fermented rice dregs was followed as the method of Tsai and Wu [13] with some modification. Twenty-five milliliter of working solution was prepared by mixing with 12.5 mL of 100 mM borax, 1.25 mL of 20% (w/w) sodium dodecyl sulfate, 20 mg of o-phthalaldehyde solution (dissolved in 0.5 mL of methanol) and 50 μL of β-mercaptoethanol and then adjusted the volume to 25 mL with deionized water. Twenty-five microliters of the fermented rice dregs was mixed with 2 mL of working solution and incubated for 2 min at ambient temperature then measured on light absorbance at 340 nm with Hybrid Multi-Mode Reader (Synergy™ H1, BioTek, Winooski, USA). The peptide content was quantified by L-leucine (Difco Laboratories, Sparks, MD, USA) as standard.

Determination of In Vitro ACE Inhibitory Activity

The ACE inhibitory activity was assayed by Cushman and Cheung [14] with some modification. Each 450 μL assay mixture contained...
the following components at the indicated final concentration: 100 mM borate buffer with 300 mM Sodium chloride 150 µL (pH 8.3); 15 mM HHL 150 mL; 150 µL of the fermented rice dregs and 8 µL ACE. The mixture was incubated at 37°C for 30 min. The reaction was stopped by adding 0.5 µL of 1 M HCl. The hippuric acid was extracted with ethyl acetate then removed ethyl acetate heat evaporation. The amount of hippuric acid was measured Hybrid Multi-Mode Reader (Synergy™ H1, BioTek, Winooski, USA) at 228 nm. The inhibition was calculated by the method of Tsai and Wu [13]. ACE inhibitory activity was calculated by using the following formula:

\[ \text{Inhibition (\%)} = \frac{A_c - A_s}{A_c - A_b} \times 100 \]

Where,

\( A_c = \) Absorbance of control sample (HHL + buffer + ACE)
\( A_s = \) Absorbance of sample solution (HHL + sample + ACE)
\( A_b = \) Absorbance of blank solution (HHL + buffer)

Animal Care

All animal experiments were approved by the Institutional Animal Care and Utilization Committee (IACUC) of Agricultural Technology Research Institute (ATRI), Xiangshan, Hsinchu, Taiwan and animal care was performed in compliance with the guidelines of IACUC. WKY rats and SHRs were freely fed a standard laboratory diet and the sterile drinking water and kept on a 12-h light/dark cycle at 24-27°C and 60-70% humidity using an automatic control system in the GLP Animal Laboratory, Animal Technology Laboratories, ATRI, Xiangshan, Hsinchu, Taiwan.

Detection of Blood Pressure of RDP-Administrated SHRs

6-8 weeks old WKY rats (n = 10) and SHRs (n = 35) were raised in GLP Animal Laboratory, ATRI. The RDP via L11 fermentation was dissolved in physiological saline at two doses of 50 and 200 mg/kg body weight (BW). According to blood-pressure level, 35 SHRs were randomized into 4 groups [negative control group: 5 rats; positive control group (20 mg/kg/day nifedipine administration): 10 rats; RDP low group (50 mg/kg BW RDP): 10 rats; RDP high group (200 mg/kg BW RDP): 10 rats]. On the other hand, 10 WKY rats were blank normal control group. RDP was administrated to SHRs by p.o. using disposable feeding needles (FN-9921, 20G × 1.5”; Kent Scientific, San Diego, CA, USA) once per day for 2 months. Twenty mg/kg/day nifedipine was administrated for SHRs as the same method of RDP.

The same volume and frequency of physiological saline was administrated to WKY rats by p.o. using disposable feeding needles. The blood pressure values were measured for each group once a week. The efficacy of RDP on the blood pressure was compared with each group. Following p.o. of sample, the blood pressure of rats was measured by a tail-cuff method (Model MK-2000ST; Muromachi Kikai, Tokyo, Japan) without warming rats in a chamber maintained at 38°C for 5 min. Five times at a time and take the average for the detection of blood pressure.

Statistical Analysis

Values of ACE inhibitor activity and production concentration of RDP are reported as mean ± Standard Deviation (S.D.). Values of blood pressure, body weight, and liver and kidney function indexes are represented as mean ± Standard Error of the Mean (SEM). Statistical evaluation was performed using Student’s t-test, one-way analysis of variance (ANOVA), and SAS 8.0 software. Differences between groups were considered statistically significant at *p < 0.05.

Results

The Most Ideal Fermentation Conditions of Rice Dregs

The fermentation conditions as fermentation time (h), pH value, and bacterial concentrations (CFU/mL) were screened. The fermentation time involved 0, 5, 24 and 48 h; pH value included 7.00, 6.99, 5.13, and 5.17; bacterial concentrations involved 1.0 × 10², 3.4 × 10², 3.9 × 10², and 3.6 × 10³ (CFU/mL). The most ideal fermentation condition is set at fermentation time (48 h), pH value (5.17), and bacterial concentration (3.6 × 10³ CFU/mL). According to the most ideal fermentation conditions, the concentrations of RDP (mg/mL) under three fermentation time (0, 24, and 48 h) were respectively calculated as 24.14 ± 0.09 (fermentation at 0 h), 31.79 ± 0.42 (fermentation at 24 h), and 30.62 ± 0.26 (fermentation at 48 h) (Table 2).

Table 1: Fermentation conditions of the rice dregs.

<table>
<thead>
<tr>
<th>Fermentation time (h)</th>
<th>pH</th>
<th>Bacterial concentrations (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7</td>
<td>1.0 × 10⁴</td>
</tr>
<tr>
<td>5</td>
<td>6.99</td>
<td>3.4 × 10⁴</td>
</tr>
<tr>
<td>24</td>
<td>5.13</td>
<td>3.9 × 10⁵</td>
</tr>
<tr>
<td>48</td>
<td>5.17</td>
<td>3.6 × 10⁵</td>
</tr>
</tbody>
</table>

Effect of RDP on ACE Inhibitory Activity In Vitro

The ACE inhibitory activity (%) and IC₅₀ (half maximal inhibitory concentration, mg/mL) of RDP was presented that 79.34 ± 4.61% and 15.21 mg/mL at 0 h-fermentation, 84.78 ± 7.72% and 18.75 mg/mL at 24 h-fermentation, 96.70 ± 5.85% and 15.83 mg/mL at 48 h-fermentation (Table 2). RDP production concentration and ACE inhibitory activity were dependently fermentation time. Zero h, 24 h, and 48 h-fermentation compared with each other were significantly different (p < 0.05) on RDP production concentration and ACE inhibitory activity (Table 2).
Table 2: Effects of RDP on ACE inhibitory activity at various fermentation times.

<table>
<thead>
<tr>
<th>Fermentation time (h)</th>
<th>Concentration of RDP (mg/mL)</th>
<th>ACE inhibitory activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inhibitory (%)</td>
</tr>
<tr>
<td>0</td>
<td>24.14 ± 0.09ᵃ</td>
<td>79.34 ± 4.61ᵃ</td>
</tr>
<tr>
<td>24</td>
<td>31.79 ± 0.42ᵇ</td>
<td>84.78 ± 7.72ᵇ</td>
</tr>
<tr>
<td>48</td>
<td>30.62 ± 0.26ᶜ</td>
<td>96.70 ± 5.85ᶜ</td>
</tr>
</tbody>
</table>

Abbreviation: angiotensin I-converting enzyme (ACE); half maximal inhibitory concentration (IC₅₀); rice dreg peptides (RDP); standard deviation (SD). Data were presented as mean ± SD. Different latters (a, b, and c) was presented significant difference inter-groups.

Effect of RDP on BW, Anti-hypertension, and Liver and Kidney Functions In Vivo

The once per day- p.o. experiments with RDP in 6-8 weeks old SHRs. Thirty five SHRs were randomized into 4 groups: negative control group, positive control group (nifedipine administration), RDPₙ₇ group (50 mg/kg RDP), RDPₙ₉₉ group (200 mg/kg RDP). Ten WKY rats were blank normal control group. RDG was administrated by p.o. through gavage per day for 2 months. The BW and blood pressure values were measured for each group once a week. The BW of all rats were increase continuously and were showed non-significantly different (p > 0.05) between 5 groups each other (Table 3). The blood pressure of SHRs before and after experiment in four groups were respectively 181.20 ± 5.51 mmHg; 192.20 ± 3.92 mmHg (negative control group), 170.50 ± 3.49 mmHg; 129.44 ± 3.80 mmHg (positive control group), 171.40 ± 5.29 mmHg; 167.00 ± 0.92 mmHg (RDPₙ₇ group), and 159.70 ± 6.69 mmHg; 159.70 ± 1.01 mmHg (RDPₙ₉₉ group). The efficacy of anti-hypertension of nifedipine is significantly higher than RDP (p < 0.001).

Table 3: Change of body weight in rats during RDP administration.

<table>
<thead>
<tr>
<th>Week</th>
<th>WKY rats</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal control</td>
<td>Negative control</td>
</tr>
<tr>
<td>0</td>
<td>200.07 ± 2.98</td>
<td>215.03 ± 3.04</td>
</tr>
<tr>
<td>1</td>
<td>250.61 ± 3.66</td>
<td>248.36 ± 3.05</td>
</tr>
<tr>
<td>2</td>
<td>267.20 ± 3.95</td>
<td>263.97 ± 2.15</td>
</tr>
<tr>
<td>3</td>
<td>281.75 ± 4.60</td>
<td>273.97 ± 4.90</td>
</tr>
<tr>
<td>4</td>
<td>294.04 ± 5.27</td>
<td>280.98 ± 5.71</td>
</tr>
<tr>
<td>5</td>
<td>309.72 ± 4.90</td>
<td>294.16 ± 6.84</td>
</tr>
<tr>
<td>6</td>
<td>315.21 ± 4.71</td>
<td>302.87 ± 5.03</td>
</tr>
<tr>
<td>7</td>
<td>324.31 ± 4.34</td>
<td>301.44 ± 10.72</td>
</tr>
<tr>
<td>8</td>
<td>335.00 ± 4.77</td>
<td>306.12 ± 11.89</td>
</tr>
</tbody>
</table>

Abbreviation: Wistar Kyoto (WKY); Spontaneously Hypertensive Rats (SHR); Standard Error of the Mean (SEM); Rice Dreg Peptides (RDP); Low Concentration of RDP (RDPₙ₇); High Concentration of RDP (RDPₙ₉₉). Data were presented as mean ± SEM.

The mean blood pressure in two RDP groups was significant lower than negative control group (p < 0.01 - p < 0.001) (Figure 1). The mean blood pressure in two RDP groups was significant lower than negative control group (p < 0.01 - p < 0.001) (Figure 1). Moreover, 4 indexes (GOT, GPT, BUN, and CRE) of the liver and kidney functions of RDP-administrated SHRs were located at the normal levels (Table 4).

Table 4: Effects of RDP on liver and kidney function indexes of WKY rats and SHR.

<table>
<thead>
<tr>
<th>Before</th>
<th>WKY rats</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal control</td>
<td>Negative control</td>
</tr>
<tr>
<td>GOT (U/L)</td>
<td>101.60 ± 10.92</td>
<td>92.40 ± 7.81</td>
</tr>
<tr>
<td>GPT (U/L)</td>
<td>46.40 ± 2.26</td>
<td>33.80 ± 0.73</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>20.12 ± 0.87</td>
<td>24.34 ± 1.15</td>
</tr>
<tr>
<td>CRE (mg/dL)</td>
<td>0.20 ± 0.02</td>
<td>0.18 ± 0.02</td>
</tr>
</tbody>
</table>

| After |
|-------|-------|-------|-------|-------|-------|
| GOT (U/L) | 79.00 ± 5.54 | 151.75 ± 8.02 | 91.44 ± 5.89 | 144.70 ± 5.51 | 110.90 ± 8.86 |
| GPT (U/L) | 37.00 ± 1.67 | 51.25 ± 4.75 | 38.78 ± 1.60 | 42.90 ± 1.57 | 40.30 ± 1.61 |
| BUN (mg/dL) | 17.76 ± 0.66 | 21.55 ± 0.70 | 20.30 ± 0.96 | 19.20 ± 0.57 | 22.02 ± 0.58 |
| CRE (mg/dL) | 0.24 ± 0.02 | 0.28 ± 0.02 | 0.20 ± 0.00 | 0.25 ± 0.02 | 0.23 ± 0.02 |

Abbreviation: Wistar Kyoto (WKY); Spontaneously Hypertensive Rats (SHR); Standard Error of the Mean (SEM); Glutamic-
Oxaloacetic Transaminase (GOT); Glutamic-Pyruvic Transaminase (GPT); Blood Urea Nitrogen (BUN); Creatinine (CRE); Rice Dreg Peptides (RDP); Low Concentration of RDP (RDP<sub>low</sub>); High Concentration of RDP (RDP<sub>high</sub>). Data were presented as mean ± SEM.

Figure 1: Efficacy of RDP on the anti-hypertension in vivo. Thirty-five SHRs were randomized into 4 groups [negative control group: 5 rats; positive control group (20 mg/kg/day nifedipine): 10 rats; RDP<sub>low</sub> group (50 mg/kg RDP): 10 rats; RDP<sub>high</sub> group (200 mg/kg RDP): 10 rats]. Ten WKY rats were blank normal control group.

Discussion

Functional peptides can be isolated from the natural food or their by-products. These natural food sources include mainly animal matrix as milk, egg, meat, fish, etc. and plants as cereal, wheat, rice, garlic, spinach, grapes, etc. [15-23]. These functional peptides have also been reported from various biological processes such as enzymatic hydrolysis, fermentation, and chemical synthesis [7,24-26]. At present, lots of chemical drugs have been designed to act on anti-hypertension. Although these drugs are very effective in controlling blood pressure, there are a lot of side effects associated with them. Hence, search of a novel anti-hypertensive compound with no side effects is need. Rice is a major and fundamental cereal source of energy and protein in the world. It is consumed as a staple food for about half of the world’s population in over 100 countries. Rice protein has unique nutritional and hypoallergenic properties compared to other plant-sourced protein [27-28] and is suitable as a hypoallergenic protein source to replace milk and soy infant formulas [6,28-29]. Rice manufacturing industry produces large volumes of co- and by-products, which are generally under-valued and under-utilised. Although rice-sourced by-products were cheap. However, it can be renewable to producing the abundant antioxidant and bioactive compounds/peptides [5]. Currently, rice-sourced by-products are mostly used as animal feed. These proteins of rice-sourced by-products were as a valuable source for the recovery of antioxidant peptides following whole cell or proteolytic enzyme treatments [5].

Many ACE inhibitory peptides have been characterized from food proteins such as gelatin, maize, fish, eggs, pea, and whey protein [30]. In this study, the source of RDP was rice dregs via lactic acid bacterial fermentation. This RDP is a valuable source for anti-hypertension according to the data of in vitro ACE inhibitory activity and in vivo animal experiment with SHRs. We have successfully R&D to evaluate the rice sourced by-products became the renewable bio-matrix and produced the anti-ACE and anti-hypertensive peptides (RDP). RDP with ACE inhibitory activity was mainly produced by many factories such as monosodium glutamate factory, glucose factory, fermentation industry etc. Commonly, rice dregs are severed as animal feed and they can cause environmental pollution problem. Moreover, rice dregs contain at least 65% protein content, which is higher than soybean protein content.

Therefore, rice dregs are good materials for producing the anti-hypertensive peptides [30]. In this study, ACE inhibitory peptides sourced from rice dregs has some effect on blood pressure regulation in vivo with SHRs. Additionally, Increase BW and normal liver and kidney functions of RDP-administrated SHRs were found in this study. We have found a safer, effective, and economical ACE inhibitor (RDP) for the remedy of hypertension. So, rice dregs-derived
ACE inhibitory peptides via the lactic acid bacterial fermentation are safer and more efficient substitution for human health.

**Conclusion**

Our study mainly focused on the ACE inhibitory activity and anti-hypertension efficacy of RDP. The present study indicated the RDP had significant antihypertensive efficacy and no side effects were found *in vitro* and/or *in vivo*. *Pediococcus pentosaceus* L11-fermented products (RDP) from rice dregs might serve as alternative sources of dietary health care products with good nutritional quality, safety, and protection against hypertension. Our data might contribute to further research into food derived antihypertensive compounds, meanwhile it also provides some reference for the clinical drug use of RDP in traditional Chinese medicine.

**Acknowledgement**

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