Functional potato bioactive peptide intensifies Nrf2-dependent antioxidant defense against renal damage in hypertensive rats

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ARTICLE INFO

Keywords:
Hypertension
Kidney
Potato
Functional peptide
Oxidative stress
DJ-1
Nrf2

ABSTRACT

Hypertension, which is known as a silent killer, is the second leading cause of kidney failure worldwide. Elevated blood pressure causes approximately 7.6 million deaths, which account for ~13.5% of the total deaths and will continue to rise. High blood pressure is the prime risk factor associated with complications in major organs, including the heart, brain and kidney. High blood pressure accelerates oxidative stress and thereby causes organ dysfunction through the production of reactive oxygen species. In this study, we investigated the renal-protective effects of the bioactive peptide IF from alcalase potato protein hydrolysate in spontaneously hypertensive rat kidney. Sixteen-week-old spontaneously hypertensive rats were divided into three groups (n = 6), and Sixteen-week-old Wistar Kyoto rats (n = 6) served as the control group. The rats were administered IF and captopril via oral gavage for 8 weeks and then sacrificed, and their kidneys were harvested. The kidney sections from the rats treated with IF showed restoration of the structure of the glomerulus and Bowman’s capsule. The expression levels of Nrf2-mediated antioxidants were also increased, as confirmed by 4-hydroxynonenal immunohistochemical staining. The TUNEL assay revealed a significant reduction in the number of apoptotic cells in the IF-treated groups, which was consistent with the western blot results. Thus, the bioactive peptide IF exerts potential protective effects against hypertension-associated ROS-mediated renal damage via the Nrf2-dependent antioxidant pathway along the DJ-1 and AKT axes. Hence, we speculate that IF might have promising therapeutic effects on renal damage associated with hypertension.

https://doi.org/10.1016/j.foodres.2019.108862
Received 17 June 2019; Received in revised form 22 November 2019; Accepted 23 November 2019
Available online 03 December 2019
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Abbreviations: 4-HNE, 4-hydroxynonenal; ACE, angiotensin I-converting enzyme; AKT, AKT serine/threonine kinase; APPH, alcalase potato protein hydrolysate; DJ-1, protein deglycase DJ-1; GPX4, glutathione peroxidase 4; GSK-3β, glycogen synthase kinase 3β; HO-1, heme oxygenase 1; HFD, high-fat-diet; IF, isoleucine-phenylalanine; Nrf2, nuclear factor erythroid 2-related factor 2; PI3K, phosphoinositide 3-kinase; ROS, reactive oxygen species; PRDX2, peroxiredoxin 2; SHR, spontaneously hypertensive rat; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; WKY, Wistar Kyoto rat

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1. Introduction

Hypertension is one of the main risk factors causing morbidity and mortality in modern society, and the number of patients presenting this risk factor will continue increasing to approximately 30% of the general population in 2025 (Kearney et al., 2005; Sliwa, Stewart, & Gersh, 2011). Previous research has indicated that blood pressure regulation involves many genes as well as multiple organs, such as the heart, kidney, brain, and blood vessels, multiple physiological systems, such as the cardiovascular, renal, neural, and immune system, and environmental stimulation (Touyz & Briones, 2011). The pathophysiological mechanisms leading to high blood pressure include endothelial dysfunction, arterial remodeling, vascular inflammation, an unbalanced renin–angiotensin–aldosterone system, and irregular G protein-coupled receptor signaling (Touyz & Schiffrin, 2004). Reactive oxygen species (ROS) overproduction is a major cause of oxidative stress that induces the above-mentioned pathophysiological changes and thereby results in hypertension (Touyz & Schiffrin, 2004).

Previous research has confirmed that ROS, including hydroxyl radicals, superoxide radicals, and hydrogen peroxide, have a close connection with the pathogenesis of hypertension (Qiao et al., 2016). Indeed, controlled ROS groups play essential roles in the maintenance of physiological processes, including blood pressure regulation, at the molecular level. In contrast, the unregulated production of ROS, which leads to oxidative stress, consequently results in DNA, protein and lipid damage and ultimately leads to cell injury and cytotoxicity, has also been implicated in essential hypertension (Togliatto, Lombardo, & Brizzi, 2017). Cell apoptosis is an essential process of cell death for the removal of harmful cells in normal organs. This process involves the extrinsic and intrinsic apoptotic pathways. Nevertheless, abnormal apoptosis can cause several different diseases, including inflammation, cardiovascular diseases, cancer, respiratory disorders, neurodegenerative diseases, and hypertension. Oxidative stress is a risk factor that induces abnormal apoptosis in the body. For example, oxidative stress results in activation of the mitochondrial (intrinsic) apoptotic pathway and activation of the transmembrane death receptors (such as the Fas cell surface death receptor, tumor necrosis factor-related apoptosis-inducing ligand receptor 1/2 and tumor necrosis factor receptor 1)-dependent apoptotic pathway to trigger diseases (Redza-Dutordoir & Averill-Bates, 2016). Because hypertension can be induced by oxidative stress and produces more oxidative stress, the inhibition of excess ROS production is a useful therapy for attenuating high blood pressure and maintaining the normal function of organs in the body.

In general, hypertension not only causes heart diseases but also leads to hypertensive end organ damage, such as vasculopathy, cerebrovascular damage, and nephropathy (Schmieder, 2010). The kidney is one of the organs that regulate blood pressure; however, it has been reported that hypertension can result in renal failure. In hypertension-induced renal injury, the overaccumulation of free oxygen radicals has been observed due to the reduction of antioxidative stress mechanisms, which demonstrates that hypertension-induced renal injury induced by oxidative stress plays a crucial role in hypertensive renal damage (Mennuni et al., 2014; Schmieder, 2010; Togliatto et al., 2017). Therefore, the investigation of new therapies for eliminating hyper-tensive damage activity.

Despite the many studies addressing the protective function of bioactive peptides from APPH, little has been reported about the protective function in kidney under hypertension. The aim of this study was to focus on the potential protective effects and the regulatory mechanism of the bioactive peptide IF from APPH on hypertension-induced renal damage in a spontaneously hypertensive rat (SHR) model. We thus performed an eight-week experimental study with SHRs treated with the functional bioactive peptide IF or captopril, a well-known angiotensin-converting-enzyme inhibitor. The dosages were fixed based on the preliminary study on the dosages in vitro models and in animal models. The dosages did not show any toxic effects (Data not shown).

The findings indicated that potato bioactive peptide IF not only was an ACE inhibitory peptide in SHR but also had protective effect in kidney under hypertension.

2. Materials and methods

2.1. Materials

All of the chemicals used in this study were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. The potato bioactive peptide IF was commercially synthesized at the N terminus and purified by RP-HPLC to 98% purity by DGG Peptides Co., Ltd. (Hangzhou, Zhejiang, China). Primary antibodies, including p-Akt5473 (Cat#sc-7985, Santa Cruz Biotechnology, Santa Cruz, CA, USA), p-Akt2860 (Cat#9275, Cell Signaling Technology, Danvers, MA, USA), AKT (Cat#s-5298, Santa Cruz Biotechnology), pBad312 (Cat#9296, Cell Signaling Technology), Bad (Cat#s-8044, Santa Cruz Biotechnology), Bak (Cat#s-7873, Santa Cruz Biotechnology), Bax (Cat#s-526, Santa Cruz Biotechnology), Bcl-2 (Cat#s-7382, Santa Cruz Biotechnology), Bcl-xL (Cat#s-8392, Santa Cruz Biotechnology), caspase-3 (Cat#9662, Cell Signaling Technology), cleaved caspase-3 (Cat#9664, Cell Signaling Technology), caspase-8 (Cat#AB1879, Merck Ltd., Darmstadt, Germany), caspase-8 (Cat#s-56070, Santa Cruz Biotechnology), caspase-9 (Cat#9506, Cell Signaling Technology), catalase (Cat#s-271803, Santa Cruz Biotechnology), cytchrome C (Cat#sc-13560, Santa Cruz Biotechnology), DJ-1 (Cat#5933, Cell Signaling Technology), GAPDH (Cat#s-32233, Santa Cruz Biotechnology), GPX4 (Cat#14432-1-AP, Thermo Fisher Scientific Inc., Waltham, MA, USA), p-GSK-3β (Cat#sc-373800, Santa Cruz Biotechnology).
Biotechnology), GSK-3β (Cat#sc-9166, Santa Cruz Biotechnology), HO-1 (Cat#sc-136961, Santa Cruz Biotechnology), 4-hydroxynonenal (Cat#ab46545, Abcam, Cambridge, UK), Keap1 (Cat#sc-33569, Santa Cruz Biotechnology), Mcl-1 (Cat#sc-12756, Santa Cruz Biotechnology), Nrf2 (Cat#ab89443, Abcam), p-Nrf2 (Cat#ab76026, Abcam), PRDX2 (Cat#ab59539, Abcam), PGC-1α (Cat#sc-518025, Santa Cruz Biotechnology), SOD1 (Cat#sc-8637, Santa Cruz Biotechnology), and SOD2 (Cat#sc-13134, Santa Cruz Biotechnology), were added to the membranes for the recognition of specific proteins. The secondary antibodies were purchased from Sigma-Aldrich (St. Louis, MO, USA). The protease inhibitor cocktail (Cat# S8830) and phosphatase inhibitor cocktail 2 (Cat# P5726) were purchased from Sigma-Aldrich.

2.2 Animal experiments and treatments

All the animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of China Medical University (Taichung, Taiwan) through the IACUC-100-12 protocol. Healthy Wistar Kyoto rats (WKY) (n = 6, male) and SHRs (n = 18, male) aged four months were purchased from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). All the rats were bred in a room maintained at 24 ± 2 °C and 55 ± 10% humidity with a 12-hour light cycle and were provided with standard laboratory chow and water. Eighteen male SHRs were randomly divided into three groups (each with n = 6): hypertension negative control SHRs (SHR), bioactive peptide IF-treated group SHRs (IF), and SHR captopril-treated SHRs (Cap). Six WKY rats comprised a normal negative control group (WKY). PBS was used as the vehicle in the WKY and SHR control groups. The SHRs were confirmed to have hypertension through measurements of the blood pressure prior to treatment. Captopril, a clinical drug, was used in this study as a positive control due to its role in inhibiting angiotensin-converting enzyme. The SHRs were treated with the bioactive peptide IF (10 mg/kg body weight) or captopril (5 mg/kg body weight) three times a week for eight weeks via oral gavage. After eight weeks of treatment, all the rats were euthanized in a pre-determined protocol. The tissue sections were then incubated with the primary antibody (4-hydroxynonenal in 1% horse serum) for 1 h and washed with PBS. All the slides were individually stained with horseradish peroxidase-conjugated avidin biotin complex (ABC) from the Vectastain Elite ABC Kit and NovaRED chromogen (Vector Laboratories, Burlingame, CA, USA) and counterstained with hematoxylin. Photomicrographs were obtained using an OLYMPUS® BX53 microscope.

2.3 Paraffin tissue section

Kidney tissue sectioning was performed following previously described methods with slight modifications (Chiu, Yang, Chiang, Chou, & Yang, 2014). The collected kidney tissues from each group were immersed in 10% formalin for two days, dehydrated by consecutive immersions in alcohols and fixed with paraffin wax. Paraffin-embedded tissue blocks were sectioned into 0.2-μm-thick slices.

2.4 Hematoxylin and eosin (HE) staining

The tissue sections were deparaffinized with xylene and rehydrated through a graded alcohol series. All the slices were stained with hematoxylin and eosin and then washed with water. Each slide was dehydrated through a graded alcohol series and soaked twice in xylene. Photomicrographs were obtained using an OLYMPUS® BX53 microscope (Olympus® Corporation, Shinjuku-ku, Tokyo, Japan).

2.5 Masson’s trichrome staining (MS)

The tissue sections were deparaffinized with xylene and rehydrated through a graded alcohol series. The tissue slides were incubated with Masson’s trichrome dye for 5 min and then washed with water. Each slide was dehydrated through a graded alcohol series and soaked twice in xylene. Photomicrographs were obtained using an OLYMPUS® BX53 microscope.

2.6 Immunohistochemical staining (IHC)

The tissue sections were deparaffinized with xylene and rehydrated through a graded alcohol series. All the slides were incubated with permeabilization solution and blocking buffer and then washed with PBS. The tissue sections were then incubated with the primary antibody (4-hydroxynonenal in 1% horse serum) for 1 h and washed with PBS. All the slides were individually stained with horseradish peroxidase-conjugated avidin biotin complex (ABC) from the Vectastain Elite ABC Kit and NovaRED chromogen (Vector Laboratories, Burlingame, CA, USA) and counterstained with hematoxylin. Photomicrographs were obtained using an OLYMPUS® BX53 microscope.

2.7 TUNEL assay for apoptosis

The paraffin wax from all of the kidney tissue slides was removed with xylene, and the slides were then rehydrated through a graded alcohol series, incubated with proteinase K (2 μg/ml), washed in phosphate-buffered saline, incubated with permeabilization solution and blocking buffer, and then washed with PBS. The terminal deoxynucleotidyl transferase and fluorescein isothiocyanate-dUTP apoptosis detection kit (Roche Applied Science, Indianapolis, IN, USA) was utilized for 60 min at 37 °C. Bright-green light at 450-500 nm was used to detect the number of apoptotic nuclei. The tissue sections were also stained with 0.1 mg/ml 4,6-diamidino-2-phenylindole (DAPI) and detected by UV light at 340-380 nm. Photomicrographs were obtained using an Olympus® CKX53 microscope. All counts were performed by at least three independent individuals in a blinded manner.

2.8 Tissue protein extraction and western blotting

Kidney tissue extracts from each group were homogenized with lysis buffer (0.05 M Tris-HCl, pH 7.4, 0.15 M NaCl, 0.25% deoxycholic acid, 1% NP-40, and 1 mM EDTA, protease inhibitor cocktail (Cat# S8830, Sigma-Aldrich), phosphate inhibitor cocktail 2 (Cat# P5726, Sigma-Aldrich) at a ratio of 100 mg tissue to 1 ml of lysis buffer. The homogenates were placed overnight at −80 °C and then centrifuged at 13,000 rpm for 40 min at 4 °C (Schäfer & Kaufmann, 1999). The supernatants were collected, and the protein product concentrations from

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**Fig. 1.** Schematic of the investigation of functional bioactive peptide IF.
the kidney tissue extracts were assessed using the Lowry protein assay method. The protein concentration of the samples was calculated using Excel (Microsoft Corporation, Redmond, WA, USA). Aliquots of protein from each tissue sample were mixed with appropriate amounts of 5× loading dye, and the mixtures were then heated for 5 min at 95 °C. Western blotting was performed using previously mentioned methods (Liu et al., 2019; Wang, 2014; Wu et al., 2019). The proteins were electrophoretically separated by 8%, 10%, or 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (GE Healthcare UK, Ltd., Amersham, UK). The membranes were incubated with blocking buffer containing 5% nonfat milk (20 mM Tris-HCl (pH 7.6), 150 mM NaCl, and 0.1% Tween-20) for 1 h to block nonspecific protein binding and incubated with primary antibodies in blocking buffer overnight at 4 °C for conjugation with specific proteins. The membranes were then incubated with horseradish peroxidase-labeled antibodies, and blot images were obtained using a Fuji LAS 3000 imaging system with the ECL substrate. The data were quantified using ImageJ software from the NIH (Bethesda, MA, USA). The protein expression data were normalized to the GAPDH expression levels.

2.9. Statistical analysis

The statistical analyses were performed with GraphPad Prism software (version 5.0, CA, USA). The results are shown as the means ± standard deviation from each independent experiment. The data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test to determine the significance of the differences between the individual means. In all cases, *P < 0.05 was considered to indicate significant, and **P < 0.01 and ***P < 0.001 were considered to indicate highly significant.

3. Results

3.1. Administration of the bioactive peptide IF rescues the glomerulus in hypertension-promoted kidney damage

First, kidney tissue slides from each group were examined to assess the protective effect of the bioactive peptide IF on hypertension-induced kidney injury. Photomicrographs of HE-stained kidney tissue slides showed that the nucleus number and glomerulus size of the SHR group were significantly reduced (P < 0.001) compared with the WKY group, but these decreases were restored in IF and Cap groups (Fig. 2A). Similarly, photomicrographs of the slides stained with Masson’s trichrome showed that the WKY, IF, and Cap groups exhibited less fibrosis compared with the SHR group (Fig. 2B-2C). The structure of the glomerulus and Bowman’s capsule also showed obvious gaps in the SHR group (Fig. 2A and 2C). These results showed that the structure of the glomerulus was rescued after treatment with the bioactive peptide IF and illustrated the potential protective effects of the bioactive peptide IF in hypertension-damaged kidney.

3.2. The renal cell survival mechanism is improved by administration of the bioactive peptide IF to a hypertensive stress rat model

To assess the molecular regulators involved in renal cell survival and apoptosis, we subsequently assessed various biomarkers related to cell death and survival by western blotting. The survival biomarkers Bcl-xL and Bcl2 showed high expression levels in all groups, except the SHR group (P < 0.001), and their inhibitor Bad (BCL2-associated agonist of cell death) had similar expression levels in all the groups but showed higher phosphorylation in IF group (P < 0.001) (Fig. 3A-3B, Figure S1). The expression of Bax and cytochrome c, which are downstream of Bcl-xL/Bcl2, was reduced in the IF group (P < 0.001 and P < 0.05), but surprisingly, no change in Bak expression was observed (Fig. 3B, Figure S1). Mcl1 expression was also higher in the IF group (P < 0.001) (Fig. 3A). Both extrinsic (caspase-8, P < 0.01) and intrinsic (caspase-9, P < 0.05) apoptotic pathways were scaled down by treatment with the bioactive peptide IF (Fig. 3C). Activated caspase-3 was also impeded by the bioactive peptide IF (Fig. 3C, P < 0.001). Similarly, the analysis of DNA damage in the kidney of all the groups through the TUNEL assay for apoptosis revealed that the positive signal was reduced in the IF group compared with the SHR group (P < 0.01) (Fig. 3D, Figure S2). All these data suggested that supplementation with the bioactive peptide IF induced renal cell survival and reduced apoptosis in a hypertensive rat model.

3.3. The antioxidative stress ability of the hypertension-injured kidney is enhanced by the bioactive peptide IF

Based on the above-described results, we subsequently assessed the potential mechanism through which the bioactive peptide IF protects renal cells in a hypertensive rat model. The expression levels of various antioxidative stress proteins were measured. Interestingly, most proteins that act against oxidative stress, including heme oxygenase 1 (HO-1) (P < 0.01), glutathione peroxidase 4 (GPX4) (P < 0.05), superoxide dismutase 1 (SOD1) (P < 0.001), superoxide dismutase 2 (SOD2) (P < 0.001), and peroxiredoxin 2 (PRDX2) (P < 0.05), but not catalase, were heightened in the kidney tissues of the IF group compared with the SHR group (Fig. 4A-4B, Figure S3). Similarly, analysis of the histological sections revealed that the WKY and IF groups generated decreased amounts of the lipid peroxidation biomarker 4-hydroxynonenal (4-HNE), whereas the SHR and Cap groups showed more 4-HNE on the slides (Fig. 4C, Figure S4). Expression of the 4-HNE-modified protein was also increased in the SHR group (P < 0.05) (Fig. 4C). According to these data, bioactive peptide IF inhibited ROS production in hypertension-injured kidney by inducing antioxidative stress proteins.

3.4. The Nrf2 transcription factor is utilized to switch on antioxidative stress activity during bioactive peptide IF treatment

Because cellular antioxidative stress is involved in the nuclear factor erythroid 2-related factor 2 (Nrf2) signal pathway (Padiya et al., 2014; Zhang et al., 2017), we investigated the role of Nrf2 in the bioactive peptide treatment group. As anticipated, the Nrf2 expression level was substantially increased after treatment with the bioactive peptide IF (P < 0.001), and the level of phosphorylated Nrf2 was also increased (P < 0.01). Intriguingly, PGClα, a transcriptional coactivator, was also increased after bioactive peptide IF treatment (P < 0.05) (Fig. 5A). These results indicated that the bioactive peptide IF could control antioxidative protein expression by activating the Nrf2 transcriptional regulator. Furthermore, to demonstrate the possible pathway through which the bioactive peptide IF regulates Nrf2, several Nrf2 regulators were analyzed. The data showed that Nrf2 activator deglycase DJ-1 (DJ-1) was enhanced (P < 0.001), but Nrf2 inhibitor Keap1 had similar expression levels in all groups (Fig. 5B, Figure S5). In the IF group, phosphorylated AKT (P < 0.001) was also increased to inactivate Nrf2 inhibitor glycogen synthase kinase 3β (GSK-3β) by GSK-3β phosphorylation (Fig. 5C). We concluded that the induction of antioxidative activity by the bioactive peptide IF might occur via the DJ-1 axis and the AKT signaling pathway to regulate Nrf2 activity and prevented Nrf2 degradation by AKT activation and GSK-3β phosphorylation in hypertension-induced kidney damage.

4. Discussion

Hypertension triggers an ROS production/clearance imbalance in cells to induce oxidative stress conditions, and the imbalance rate translates into increased ROS bioavailability and oxidative stress-induced cell apoptosis (Redón et al., 2003; Togliatto et al., 2017). The results obtained in this study showed that treatment with the bioactive
peptide IF protected renal cells against high blood pressure-associated oxidative stress by enhancing the expression of proteins that act against ROS to improve renal cell survival in a hypertensive rat model. Treatment with the bioactive peptide IF maintained the structure of the glomerulus compared with the SHR control group. Furthermore, the bioactive peptide IF reactivated the compensatory DJ-1 and AKT survival pathways to improve Nrf2 activation and inhibit Nrf2 degradation and thereby stimulated oxidative stress defense mechanisms to inhibit renal cell apoptosis.

Our present findings on a bioactive peptide from potato protein hydrolysate align with our previous findings. In an aging Sprague-Dawley rat model, HFD feeding led to obesity in the rats and enhanced the expression levels of cellular apoptosis and fibrosis proteins; however, APPH can considerably ameliorate liver apoptosis and fibrosis by reducing hepatic lipid accumulation and improving the cell-protective pathway to resist HDF-induced hepatocyte apoptosis (Chiang et al., 2016). Similar results were observed in the senescence-accelerated mouse prone 8 aging mouse model. Intriguingly, liver steatosis and inflammatory damage caused by HDF-induced nonalcoholic fatty liver disease can be alleviated by supplementation with potato protein hydrolysate or DKTNKPVIF peptide. Liver steatosis in HDF-fed aging mice is decreased by induction of the AMPK signaling pathway (Dumeus et al., 2018). Simultaneously, the proinflammatory reaction is limited in HDF-fed aging mice by promoting AMPK signaling through APPH supplementation and DKTNKPVIF peptide treatment (Dumeus et al., 2018). Furthermore, APPH exerts cardioprotective effects. For instance, HFD-induced cardiac damage is diminished by APPH supplementation in hamster models and HFD-fed aging rats (Hu et al., 2015; Zaheer & Akhtar, 2016). APPH administration increases sirtuin 1 expression to enhance PGC-1α transcriptional activity and thereby protects cells against apoptosis (Huang et al., 2015). The AKT signaling pathway is also induced to suppress HDF-induced FoxO3a phosphorylation and thus provide effective cardiac protection against obesity damage (Huang et al., 2015). Transforming Growth Factor Beta induces cardiac hypertrophy, and fibrosis is also reduced by APPH treatment (Zaheer & Akhtar, 2016). Bioactive functional peptides synthesized from APPH act against hyperglycemia by inhibiting NFATC3 and the MAPK cascade of cardiomyocyte hypertrophy and further suppressing p38 activation in the H9c2 cell model. These peptides also protect the biological activity of H9c2 cells under high-glucose conditions by decreasing the expression of apoptotic proteins and increasing the expression of survival and anti-apoptotic proteins (Asokan et al., 2018). However, no previous study has focused on antioxidant defense in the hypertensive kidney triggered by the bioactive peptide IF.

Several previous studies confirmed that short bioactive peptides purified from natural foods act as antihypertensive agents to reduce angiotensin I-converting enzyme (ACE), a key enzyme to produce Angiotensin II and cause blood pressure increase (Daskaya-Dikmen, Yucetepe, Karbancioglu-Guler, Daskaya, & Ozcelik, 2017; Fu, Alashi, Young, Therkildsen, & Aluko, 2017; Martin & Deussen, 2019; Xue et al., 2018). Generally, the bioactivities of ACE inhibitory peptides are demonstrated by in vitro ACE inhibitory activity assay. In recent years,
Hypertension animal models are also used to confirm antihypertensive effects of ACE inhibitory peptides (Daskaya-Dikmen et al., 2017; Martin & Deussen, 2019). For example, tri-peptides (VPP and IPP) from miso-processed food show antihypertensive activity in SHRs (Tsai, Lin, Pan, & Chen, 2006). Otherwise, combination of ACE inhibitory peptides and other chemical reagent are also presented anti-inflammatory and better antihypertensive effects in vitro and SHR model (Luo et al., 2017).

In previous research, dipeptides: isoleucine-tryptophan, isoleucine-tyrosine and isoleucine-phenylalanine significantly reduce angiotensin I-converting enzyme (ACE) activity in vitro (Martin & Deussen, 2019; Martin et al., 2015). In present study, isoleucine-phenylalanine was used, not only because of our previous research, but also it had been found that the blood pressure in SHRs were decrease and heart function was repaired after bioactive peptide IF treatment (Data not shown). Therefore, we considered that potato bioactive peptide IF to reduce hypertension is by inhibition of ACE activity and reduced ROS generation in SHRs. Previous researches focus on ACE inhibition by bioactive peptide IF. Less study is related to other benefits from bioactive peptide IF in hypertensive animal model. Therefore, we demonstrated that bioactive peptide IF not only reduced hypertension, but also induced endogenous antioxidant mechanism in kidney under hypertension conditions. The western blot analysis of kidney samples revealed higher expression levels of endogenous antioxidant proteins, including SOD1, SOD2, PRDX2, HO-1, and GPX4, and these findings suggest that administration of the bioactive peptide IF induces the expression of antioxidant proteins to reduce oxidative damage by limiting ROS overproduction and weakening cell apoptosis and thereby improve renal cell survival. Based on the findings, it also can be concluded that the bioactive peptide IF can potentially be used as a functional bioactive peptide to decrease hypertensive damage in the kidney.

Interestingly, captopril is an angiotensin-converting enzyme inhibitor. Although captopril may have antioxidant activity in the kidney,
higher expression levels of some antioxidant proteins, such as GPX4 (P < 0.01), PRDX2 (P < 0.05), SOD1 (P < 0.01), and SOD2 (P < 0.001), were obtained with the bioactive peptide IF compared with captopril treatment (Fig. 4A–4B) (Vahidirad, Arab-Nozari, Mohammadi, Zamani, & Shaki, 2018). These results suggest that the bioactive peptide IF might have a greater ability to activate antioxidant defense in the kidney than captopril.

The antioxidant transcription factor Nrf2 is a ubiquitous regulator that tightly regulates the expression of antioxidant genes in response to both exogenous and endogenous ROS stress. Under conditions of ROS balance, the cytoplasmic repressor protein Keap1 ubiquitinates Nrf2 via the Keap1-Nrf2 complex to tag it for degradation by the proteasome. In contrast, under oxidative stress conditions, Nrf2 is separated from Keap1 and activates downstream cytoprotective genes (Deshmukh, Unni, Krishnappa, & Padmanabhan, 2017). To regulate the Keap1-Nrf2 complex, DJ-1 plays a main role in cellular antioxidant responses and upregulates the level of DJ-1 to activate the PI3K-Akt-Nrf2 signaling pathway for neuroprotection (Zhang et al., 2017). This finding implies the importance of amino acids in cellular antioxidative activity. Potato bioactive peptide IF contains abundant levels of isoleucine and phenylalanine, and previous studies have demonstrated the antioxidative characteristics of these two amino acids. For instance, isoleucine restores SODs to restrict ROS generation in exercised rats (Moura, Lollo, Morato, Risso, & Amaya-Farfan, 2017). In a fish model, dietary isoleucine enhances the fish immune response and controls the Nrf2-dependent antioxidant status and cytokine, TOR and 4E-BP gene expression to improve the fish quality (Gan et al., 2014; Zhao et al., 2013). Phenylalanine reduces the increased blood pressure and decreased vasodilation observed in di-amino-hydroxy-pyrimidine-treated rats (Mitchell, Dorrance, & Webb, 2004). One of its metabolites, tyrosine, also ameliorates oxidative damage through ferryl heme...
Fig. 5. The Nrf2 antioxidative pathway is induced by the bioactive peptide IF in SHRs. The bioactive peptide IF likely activates the Nrf2-dependent antioxidative pathway via the DJ-1 and AKT pathways. (A) The expression levels of total Nrf2, phospho-Nrf2, and PGC-1α in kidney samples were observed by western blotting. *P < 0.05, **P < 0.01, and ***P < 0.001. (B) Regulators related to the Nrf2 antioxidative pathway in kidney samples were analyzed by western blotting. +++P < 0.001 (C) The expression of AKT, p-AKT, p-GSK-3β, and GSK-3β in the kidney was measured by western blotting. +++P < 0.001 and +++P < 0.001. The data are expressed as the means ± standard deviation (SD).

Fig. 6. The schematic diagram of DJ-1 and AKT pathway regulating Nrf2 dependent signaling pathway by potato bioactive peptide IF in hypertension-associated oxidative renal damage. (A) Oxidative stress is increased and cause renal damage under hypertension. (B) Potato bioactive peptide IF reduces hypertension and induces Nrf2 antioxidative pathway via DJ-1 and AKT axes to decrease renal cell apoptosis.
reduction in vitro and in vivo (Garibotto, Tessari, Verzola, & Dertencor, 2002; Lu et al., 2014). All these studies support our data and suggest that our functional bioactive peptide IF has antioxidative ability in hypertensive rat model. However, because our results demonstrate that treatment with the functional bioactive peptide IF relieves antioxidative stress via both the DJ-1 and AKT axes to activate the Nrf2-dependent antioxidant defense mechanism in the kidney (Fig. 6), the findings also provide a new approach for the future application of the functional bioactive peptide IF to relieve hypertensive renal diseases.

In previous research, it has been shown that IF reduces angiotensin I-converting enzyme (ACE) activity in vitro (Martin & Deussen, 2019; Martin et al., 2015). However, the bioavailability of IF from digestive system in body was not clearly understood. Manoharan et al suggests that short peptide sequences have been known to be easily absorbed into the circulation and retain their activity (Manoharan, Shuib, & Abdullah, 2017). Since potato bioactive peptide IF is a dipeptide, we hypothesize that it can be absorbed into the circulation and retain their activity. Nevertheless, we tend to that the absorption, distribution, metabolism, and excretion of potato bioactive peptide IF in body will need further research which we will consider in our future studies.

5. Conclusion

Potato bioactive peptide IF not only is an ACE inhibitory peptide to reduce hypertension in SHRs, but also can regulate hypertension-associated oxidative stress in SHRs. Our data showed that antioxidative stress proteins, including glutathione peroxidase 4 (GPX4), heme oxygenase 1 (HO-1), superoxide dismutase 1 (SOD1), superoxide dismutase 2 (SOD2), and peroxiredoxin 2 (PRDX2), were induced by the bioactive peptide IF in SHR kidney. Cell survival signal was enhanced and cell apoptosis was limited in the kidney of SHRs. It suggests that the bioactive peptide IF is a potential renal-protective ingredient capable of counteracting oxidative stress and thus maintaining the ROS balance in SHR kidney to reduce hypertensive damage. This study provides the first demonstration that treatment with the bioactive peptide IF provides physiological protection to the kidney of rats against oxidative stress related to high blood pressure. Importantly, although the clinical medication captoril displays antioxidative activity in the kidney, the bioactive peptide has a better ability to trigger an antioxidative mechanism in the kidney. Overall, potato bioactive peptide IF can potentially be developed into an alternative promising therapy for the amelioration of ROS-induced renal damage in hypertensive rat model and might be instrumental for the development of therapeutic strategies against renal injuries in patients suffering from hypertension.

CRediT authorship contribution statement

Bruce Chi-Kang Tsai: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. Dennis Jine-Yuan Hsieh: Data curation, Validation, Visualization. Wan-Teng Lin: Conceptualization, Resources. Shanmugam Tamilselvi: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. Cecilia Hsuan Day: Resources. Tsung-Jung Ho: Formal analysis, Investigation. Ruey-Lin Chang: Writing - original draft. Vijaya Padma Viswanadha: Writing - review & editing. Chia-Hua Kuo: Methodology, Project administration, Supervision. Chih-Yang Huang: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgments

This study was financially supported in part by grants from Asia University and China Medical University Hospital (ASIA-104-CMUH-07) and in part by grants from China Medical University and Asia University (CMU105-ASIA-09). We thank American Journal Experts for the English editing assistance and the help provided by Dr. Farheen Khan and Dr. Marthandam Asokan Shibu.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.108862.

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