Advance research in functional and healthy food from animal products antihypertensive peptides derived from meat protein hydrolysates

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ABSTRACT: Meat is a good source of effective peptides in preventing and reducing chronic life-style related disease (CLSRDs) such as hypertension. Lack in crucial nutrients consumption such as protein of plant or animal origin along with abnormalities in carbohydrate and fat metabolism may underlie the aetiology of the clinical course of hypertension. Functional food derived from meat rich in those nutrients may utilize physiological function of peptides as well as improve digestion and metabolism carbohydrate and fats, thus lowering blood pressure and normalize associated biochemical and histopathological changes in man body. Edible meat comes in top of the most valuable animal products that the proteolytic action of meat muscle tremendously generates a profound number of multi-amino acid peptides, some of them with a strong relevant antihypertensive activity.

INTRODUCTION

The nutritional and functional properties of food proteins have been investigated for many years. The nutritional quality of a protein depends on its amino acid content and on the physiological utilization of specific amino acids after digestion and absorption (Friedman 1996).

Bioactive peptides have been detected in many different food source, with milk protein being the most commonly known source (Jelen, & Lutz, 1998). Among the different classes of bioactive peptides, the antihypertensive peptide are the best known (Clare, & Swaisgood, 2000). The main group of the antihypertensive peptides corresponds to the inhibitors of angiotensin I-converting enzyme (ACE).

Recently, specific peptides are considered as inhibitors of ACE, which are potentially used as pharmaceuticals to treat hypertension (Vercruysse et al., 2005). Many ACE inhibitory peptides from foods have been reported (Arihara et al. 2001, Kawamura et al. 1992), but rarely have the inhibitory mechanisms of these peptides been examined. Fujita et al. (2000) and Yokoyama et al. (1992) reported the ACE resistance of ACE inhibitory peptides from the enzymatic digests of chicken breast muscle and ovalbumin.

They tentatively classified these peptides into three categories: inhibitory peptides type, substrate peptides type and pro-drug peptides type. In all of the reports described above, it was emphasized that the resistance of the ACE inhibitory peptide against ACE itself, or against digestive enzymes, was a prerequisite for their action *in vivo*. Substrate type peptides are those which show a decrease in ACE inhibitory activity after being cleaved by ACE. The inhibitory activity of the inhibitor type peptide is not significantly affected by ACE cleavage. The pro-drug type peptides are those which show an increase in ACE inhibitory activity after ACE cleavage. It has also been reported that the substrate type peptides do not affect the blood pressure of spontaneously hypertensive rat (SHR) but the inhibitor and pro-drug type peptides produce a reduction in blood pressure values (Muguruma, Ahhmed, & Kawahara, 2008). Also in the same study, on the basis of this classification, we discovered a peptide named A5, which was also defined as a substrate type and another peptide was identified as pro-drug type peptide and called M6, where they also suggested that M6 has greater antihypertensive activity than A5 *in vivo* (Muguruma et al., 2009).

The aim of this article is to present the possible functions of some peptides sourced from animal meat on blood pressure.

ANGIOTENSIN CONVERTING ENZYME MECHANISM AND BLOOD PRESSURE

Angiotensin converting enzyme (ACE) is a dipeptidyl peptidase transmembrane bound enzyme. A soluble form of ACE in plasma is derived from the plasma membrane-bound form by proteolytic cleavage of its COOH-terminal domain. Mainly angiotensin converting enzyme is a tool degrades bradykinin and has the potential to cut any available peptides, a potent vasodilator, and other vasoactive peptides (angiotensin-I). It is a circulating enzyme that participates in the renin-angiotensin system, by cutting tow amino acids of the substrate (angiotensin-I). That action of this enzyme goes beyond liberating angiotensin-II from angiotensin-I or inactivating bradykinin (Ervin, 1990) (Fig. 1). The function of angiotensin-II is to cause constriction of arteries, thereby elevating blood pressure as blood flows in a narrower paths bloodstream. ACE inhibitors lower blood pressure by inhibiting the formation of angiotensin-II, thus relaxing the arteries.

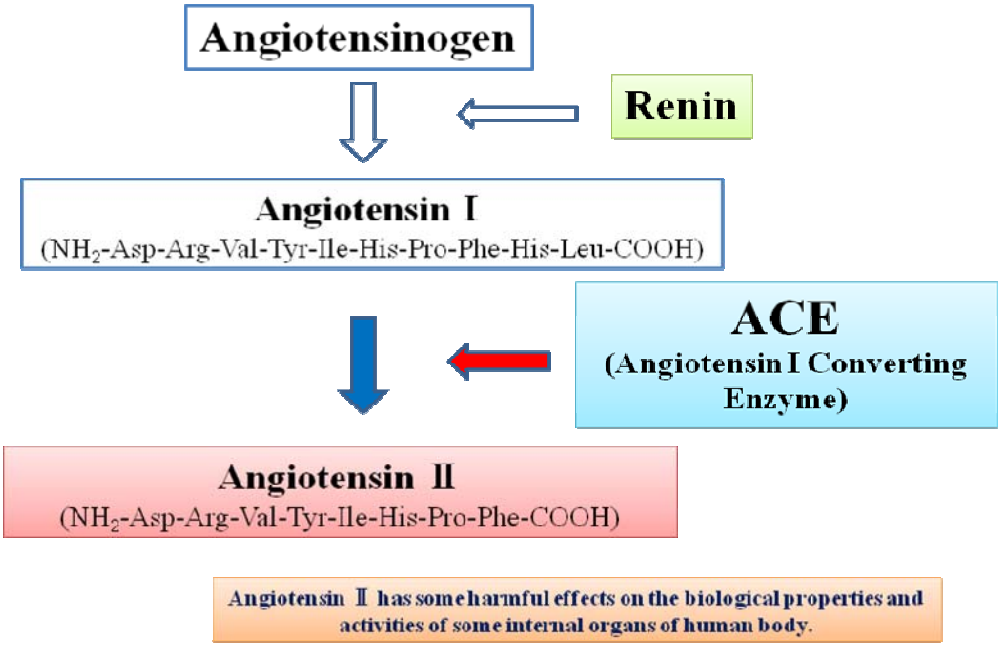

Figure 1. Renin-angiotensin system for blood pressure regulation

As the roles of biologically active peptides of meat being absorbed in man body, start to be active, they meet competitively (depends of their types) with ACE, in which they block and inactivate ACE. Inactivation of ACE generates stability for the angiotensin-I, the more angiotensin-I stable, the more the blood pressure normal. The peptides that play inhibitor roles compete with ACE to interlock in the same place that ACE cleave the angiotensin. As the competition is high, the ACE contents the condition whereas the arteries would not be shrinkage.

PROCEDURE TO EXAMINE ACE INHIBITORY ACTIVITY

With slight improvement in the procedure that we used in previous studies (Katayama et al., 2003a; 2003b, 2004; 2007; 2008; Muguruma et al., 2009), ACE inhibitory activity was measured according to the method of Cushman and Cheung (1971). The mechanism of such reaction in that protocol is counted on liberation of hippuric acid from hippuryl-L-Histidyl-L-Lucine (His-His-Leu) that basically stimulated by ACE.

A filtered sample contained biologically active peptides (6µl) is mixed with 50µl of 7.6mM His-His Leu as substrate contains 100 mM sodium borate buffer (pH 8.3) and 608 mM NaCl and then pre-incubated at 37^{o}C for 5 min. With view to initiate the reaction, a 20µl of ACE (60 miliunits/ml of rabbit lung) must be added in a buffer contains 0.25M sodium borate buffer (pH 8.3) followed by

incubation the mixture at 37 °C for 30 min. The reaction always terminated by adding 554µl of 0.1N HCl except in the case of blank samples, that have always to be treated with the same amount of HCl but before the pre-incubation step. Then the Hippuric acid liberates by ACE is always extracted by adding 1.5ml of ethyl acetate followed by vigorous shaking of the mixture for 2 min. After centrifugation at 3,000 rpm for 20 min, the process is then followed by collecting 1 ml ethyl acetate (upper layer). The collected layer of ethyl acetate was then evaporated at 100°C for 10 min. The hippric acid was then dissolved in 1 ml of 1 M NaCl and its concentration determined by photometric instrument at 228nm. The concentration of ACE inhibitors required to inhibit 50% of ACE activity is defined as the actual value of IC_{50}.

MEAT PROTEIN AND HYPERTENSION

Meat contains bioactive proteins and peptides that play important role in prevention CLSRD such as blood pressure. In essential hypertension there is a metabolic defect where glucose metabolism is altered due to insulin resistance, resulting in increased tissue levels of aldehydes and oxygen-free radicals and hypertension. This metabolic defect can be corrected nutritionally by vitamin E, vitamin

Table1. IC_{50} of ACE inhibitory peptides derived from meat and seafood vertebrate specimens.

Peptide sequence	Activity & function	IC_{50}	Source		Reference
M6 (KRVIQY)	Antihypertensive (Pd)	20.3µM	Porcine myosin B	N	Muguruma et al., 2009
A5 (VKAGF)	Antihypertensive (I)	6.1 µM	Porcine actin	N	Muguruma et al., 2009
RMLGQTPTK	Antihypertensive (I)	34 µM	Porcine troponin	N	Katayama et al., 2004
VKKVLGNP	Antihypertensive (I)	28.5 µM	Porcine skeletal muscle	N	Katayama et al., 2007
EKERERQ	Antihypertensive (I)	552.5 µM	Porcine skeletal muscle	N	Katayama et al., 2008
KRQKYDI	Antihypertensive (I)	26.2µM	Porcine skeletal muscle	N	Katayama et al., 2008
IKPLNY & IVGRPRHQG	Antihypertensive (I)	43 and 2.4µM	Muscle & Actin of bonito	N	Yokoyama et al., 1992
DYGLYP & IWH	Antihypertensive (Pd)	62 & 6.9 µM	Muscle & Actin of bonito	N	Fujita et al., 1999
VLAQYK	Antihypertensive (I)	ND	Beef muscle	N	Jang & Lee, 2005
IW & LW	Antihypertensive (I)	4.7 & 17.4 µM	Salmon muscle	N	Ono et al., 2003
YL & GWAP	Antihypertensive (I)	82 & 3.86 µM	Sardine muscle	N	Matsufuji et al., 1993
PTHIKWGD	Antihypertensive (I)	ND	Tuna meat	N	Kohama et al., 1988
LKA & FQKPKR	Antihypertensive (I)	8.5 & 14 µM	Chicken	N	Fujita et al., 2000
LAP & IVGRPRHQG	Antihypertensive (I)	3.2 & 2.4 µM	Chicken	N	Fujita et al., 2000
GFHI & DFHING	Antihypertensive (I)	117 & 64.3 µg/ml	Beef muscle	Y	Jang et al., 2008
FHG & GLSDGEWQ	Antihypertensive (I)	52.9 & 50.5 µg/ml	Beef muscle	Y	Jang et al., 2008
GDLGKTTTVSNWSPPKYKDTP	Antihypertensive (I)	11.28 µM	Tuna frame protein	N	Lee et a., 2010
VVYPWTQRF	Antihypertensive (I)	66 µmol/L	Oyster	N	Wang et al., 2008
IW	Antihypertensive (I)	1.2 µM	Salmon	N	Enari et al., 2008

References are provided, but regardless of source, all peptides showed a high ACE inhibition.
ND: Not detected; Y: Yes; N: No; I: Inhibitory type, Pd: Pro-drug type

C, vitamin B6 or a diet rich in protein-containing cysteine (Vasdev, Lognerich, Sinal, 2002). We have collectively tried to explore and categorize all the important peptides that recently were identified and their amino acid sequences as well as functions were discovered. Table 1 shows the overall crucial peptides of meat and other food stuff associated with IC_{50} values (ACE inhibitors). It is obviously clear that IC_{50} of those products was great and have the potential to lower blood pressure. IC_{50} defined as the appropriate concentration of protein or peptide to inhibit 50% of the action of ACE *in vivo*. We got the lower IC_{50} and the greater peptides in which the highest reduction in blood pressure.

EVIDENCES OF MEAT PROTEINS IN REDUCING HYPERTENSION

It was reported that the proteolytic action of pork muscle DPP typically generates a good number of dipeptides, some of them with a relevant ACE inhibitory activity (Sentandreu & Toldra, 2007). Most proteins contain bioactive sequences, but those sequences are inactive within the parent proteins.

Active peptides fragments are released from native proteins only via proteolytic digestion. Once such peptides are liberated, they can act as regulatory compounds and inhibitory nutraceuticals (Arihara, 2006). Recently, many bio-functional peptides, antihypertensives, antioxidants, antimicrobials, and antithrombotic and immunomodulatory peptides have been isolated in foods. Among these, antihypertensive peptides (ACE inhibitors) are of particular interest for prevention and treatment of hypertension (Kobayashi et al., 2008).

As many scientists purified numerous meat peptides that play nutria-functional roles, in our laboratory, we have been trying in last decade to value some meat peptides of local animals (beef, pork and chicken). We obtained some crucial peptides from muscle protein hydrolysates: porcine crude myosin (Katayam et al., 2003a); porcine skeletal muscle protein (Katayama et al., 2003b); porcine troponin C (Katayama et al., 2003c; Katayama et al., 2004); porcine skeletal muscle myosin (Katayama et al., 2007); porcine skeletal muscle troponin (Katayama et al., 2008); porcine myosin B (Muguruma et al., 2009). All the purified peptides showed a great effect on blood pressure of spontaneously hypertensive rats (SHR). The *in vitro* IC_{50} of meat peptides was very low, which means even little consumption of amount of such food, interestingly that little amount lead to inhibition 50% of ACE activity. IC_{50} defined as reading of protein concentration that always has to be calculated by certain equation to inhibit 50% of the ACE activity.

Table 1 shows some of the valuable peptides that sourced from pork, chicken, sardine, tuna and salmon. Other researchers obtained ACE inhibitory peptides from different products includes marine products and meat species such as beef (Jang et al., 2005; 2007; 2008); chicken collagen (Iwai et al., 2009); tuna (Lee et al., 2010); fugu muscle (Nagai et al., 2008); salmon (Enari et al., 2008); sardine (Otani et al., 2009); chicken bone (Nakade et al., 2008); oyster (Wang et al., 2008); sardinelle by-products (Bougatef et al., 2008); also porcine skeletal muscle (Arihara et al., 2001); chicken (Fujita et al., 2000).

Figure 2-A shows the effects of nonapeptide on the blood pressure of SHR. SHR fed orally by nonapeptide, the quantity of this peptide was 10mg per 1kg of rat's weight. This peptide showed a

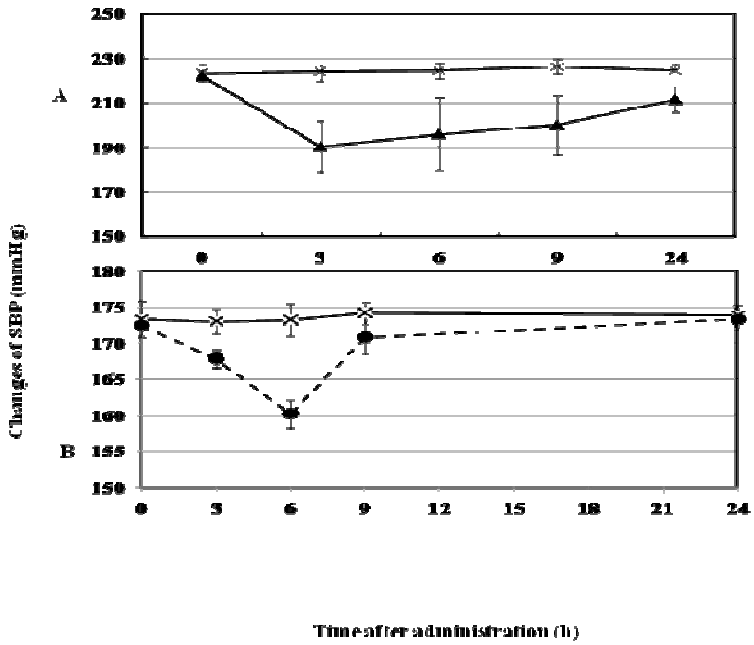

Fig. 2 Effect of single oral administration of meat-derived peptides and meat hydrolysate on SHR. Changes of systolic blood pressure (SBP) from zero time were expressed with means, and the vertical bars represent the standard deviations. Treatments were control (multiplication sign ×, distilled water) in both graphs, and nanopeptide RMLGQTPTK (triangle) in A and meat hydrolysate (labeled circles) in B. Samples administrated at a dose of 10 mg/kg body.

significant effect, specially after 3 hours, the blood pressure was decreased by a rat of 35mmHg. This nanopeptide is quite unique, because it could stabilize the blood pressure and the blood pressure contentiously remained low for almost 6 hours, from 3 to 9 hours. And at 24 hours when it compared

to the water samples it slightly decreased the blood pressure, even though we consider this peptide that it has a good ability in maintaining the blood pressure of SHR. The stability in this peptide coincide with its IC_{50}, the more ACE cut this peptide the more it become competetive, it is clear that this paptide had resisted the ACE activity inhibiting angitensin-I from being converted to angiotensin-II. Also in a study conducted by Ahhmed et al. (2009), showed that after administration of the meat hydrolyzate, the SBP decreased by 6 mmHg in 3 h and 13 mmHg in 6 h, as meat hydrolyzate has a considerable blood pressure-lowering effect (Fig. 2-B).

The concentration of angiotensin II was measured in the meat and meat hydrolysate groups of rats, and comparison of the results showed that the meat hydrolyzate had a considerable effect on the angiotensin II concentration after rats were fed a diet containing 5% meat hydrolyzate for 2 weeks (Ahhmed, & Muguruma, 2010).

The results indicate that meat hydrolyzate may contains some peptides that function as nutraceuticals and could lower the blood pressure. Administration of meat hydrolyzate led to blood-pressure-lowering effects similar to those observed in previous reports that concern about animal origin products. We monitored the blood pressure and found that it could be reduced in both the short-term and long-term perspectives by feeding rats with a diet containing meat hydrolyzate.

In hypertension, as in other progressive, CLSRDs with high mortality rates, the functional food approach offers major advantages over models with prevention tool, inhibiting rather than reducing disease and provide appropriate implementation of palliative strategies and program of medical and emotional care for the terminally ill. To determine whether a peptide is a long-term ACE inhibitor requires further investigation. This research may provide adequate evidence that meats contain a considerable number of constituents that could be utilized as functional food and nutraceuticals.

CONCLUSIONS

Results of our research along with many dozens of studies provide a body of evidence that meats possess variety of proteins consisted from large number of peptides in which showing great effect on CLSRDs. We maintain meat consumption due to recommended daily allowance, the better the health care we provide. However, excessive consumption of meats, by not follow the regular basis of consumption, may lead to unprofitable results that affect mechanisms of man body such as increasing uric acid which puts strain on the kidneys, dyspepsia and also helps obesity to be occurred. Meat hydrolyzate may also contain other constituents as a result in which gives it the opportuinty to be utilized as a functional food and nutraceutical. From the collected data of animal experiments fresh meat products would have to be considered to contain compounds that play temproally immunopharmacological role.

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