Faculty of science

Further Identification of the Effect of Bradykinin Potentiating Factor Isolated From Scorpion Venom on Irradiated White Rat

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زيادة في معرفة تأثير عامل محفز للبراديكينين المستخلص من سم العقرب علي الجرذ الأبيض المشعع

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Abstract

Scorpion venom of *Androctonus amoreuxi* contains a strong bradykinin potentiating factor (BPF) that augments bradykinin effect through enhancing its release and acts as an angiotensin converting enzyme inhibitor (ACEI). Both irradiation and stimulation of renin-angiotensin system (RAS) induce oxidative stress. Possible interruption of the RAS with an ACEI induced by BPF isolated from the scorpion, *Androctonus amoreuxi* venom or the presence of angiotensin II receptor blocker (ARB) losartan and/or γ-radiation were evaluated. The examined parameters included blood erythrocytes count (RBC), total leucocytic count (WBC), haemoglobin content (Hb) and hematocrit value (Hct) as well as, glutathione content (GSH), malondialdehyde (MDA) and advanced oxidative protein product (AOPP) of kidney homogenate besides aldosterone, sodium, potassium, chloride, calcium, urea, createnine and uric acid levels of serum.

A group of rats (70 - 80 gm each) were received i.p. injection of BPF (1μg / g body wt) twice per week for three weeks, while the other group received i.p. injection of losartan (5μg / g body wt) twice per week for three weeks. γ-Irradiation was performed at a dose level of 4Gy. All animals were examined after an investigation period of 21 days from γ-irradiation. Either BPF or losartan was performed together with irradiation.

Abstract----------

The results pointed out that irradiation discerned a significant elevation in the level of MDA, AOPP, aldosterone, sodium, urea and creatinine, and a significant drop in the haematological values (RBCs, WBCs, Hb and Hct), GSH, calcium and uric acid. Repeated injections of BPF or losartan had a beneficial result against the deleterious effect of γ-irradiation.

The present investigation clarifies comparable effects for treatment of radiation damage to the kidney through RAS by BPF as (ACEI) and losartan as (ARB). The present work adds further identification to the properties of BPF in controling of radiation damage. Therapeutic agents from natural source can be a parallel development therapy.

Contents

Introduction

Renin angiotensin system is a complex endocrine, paracrine, and autocrine system that modulates the function of the kidney (Nistala *et al*., 2009) which is classically known as a regulator of arterial pressure, which is accomplished by regulating the balance of water and sodium by their retention in the kidney leading to generating pressure. Kidneys release renin which cleaves the liver-derived angiotensinogen into Angiotensin I (Ang I). Ang I is then converted to Angiotensin II (Ang II) via the ACE in the pulmonary circulation as well as in the endothelium of blood vessels in many parts of the body (Silverthorn, 2004 and Nistala *et al*., 2009).

Beside the importance of the RAS on the circulation and other organs, the local RAS in the kidney has attracted a great attention of research in last decades. The renal RAS plays an important role in the body fluid homeostasis. All major components and key enzymes for the establishment of a local RAS have been confirmed in the kidney. In addition to renal contribution to the systemic RAS, the intrarenal RAS plays a critical role in the regulation of renal function as well as in the development of kidney disease (Xu *et al*., 2009).

Interruption of the renin-angiotensin axis with a benazepril (ACEI) or losartan (ARB) slows the progression of chronic renal insufficiency in the presence or absence of diabetes (Hou *et al*., 2006). The use of ACEI and ARB was associated with a significant improvement in renal outcome (Hou *et al*., 2007).

Inhibitors of the RAS include ACEI, ARB, and aldosterone antagonists for the treatment of hypertension particularly where kidney disease is involved.

Animal venom mostly contains characteristically small bioactive peptides. One of these components has been isolated from the Egyptian scorpions and snakes. It was referred to as BPF. It induces several physiological effects including cell division and cell differentiation (Abu-Amra, 1988; Abd-El-Rahim, 1990; Nassar *et al.,* 1990).

Bradykinin (BK) which was first discovered to have a relation with animal toxins by Rocha e Silva *et al.* (1949) is a hydrolyzed product of the low-molecular-weight kininogen by tissue kallikrein, or certain venom kallikreins (Cyr *et al*., 2001). It can induce the contraction of Guinea-pig ileum *in vitro*, and causes blood-pressure-lowering effect also (Ferreira and Henriques, 1992). Furthermore, BK has been implicated in multiple physiological processes such as control of blood pressure, contraction or relaxation of smooth muscle, inflammatory responses, and induction of hyperalgesia (Coutrue *et al*., 2001). Interestingly, it was found that there existed a factor in *Bothrops* venom which was able to potentiate the biological actions of BK (Ferreira *et al*., 1998). Moreover, this factor exhibits both BK-potentiating activity and inhibits activity to ACE which is a cytoplasmic membrane peptidase of endothelial cells responsible for the conversion of Ang I to Ang II (Murayama *et al*., 1997). ACEI appear to potentiate BK beyond blocking its hydrolysis, (Marcic *et al*., 2000).

Losartan known as a selective non-peptide antagonist blocking the angiotensin type 1 receptor (AT1). It was first discovered in 1992 by Duncia *et al*. Its chemical structure is 2-Butyl-4-chloro-1-[(2′- (1H-etrazol-5-yl) (1, 1′-biphenyl-4-yl) methyl]-1H-imidazole-5- methanol with the molecular formula of $C_{22}H_{22}N_6O$ and molecular weight of 461.01(Xu *et al*., 2009).

Irradiation of the kidneys is followed by a well-defined sequence of changes leading eventually to kidney failure. In the rat, inhibition of ACE or blockade of AT1 receptors can prevent the structural and functional changes that occur after kidney irradiation. These interventions are particularly effective between 3 and 10 weeks after irradiation (Cohen *et al*., 2002).

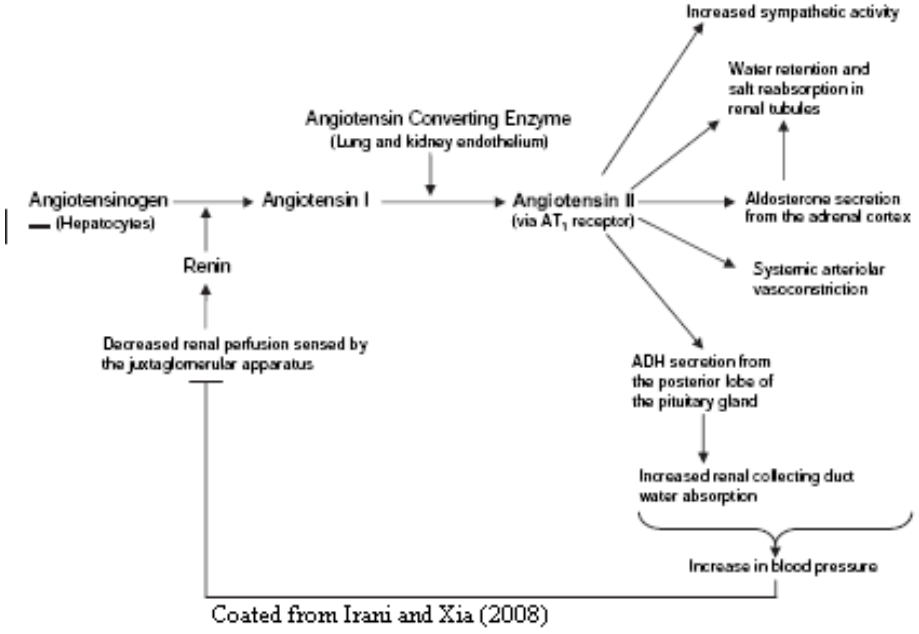
Aim of the work:

This work aims to clarify and investigate further characterization of the properties of the bioactive peptide BPF (ACEI) isolated from the venom of Egyptian scorpion *Androctonus amoreuxi* and its effect on interruption of RAS and kidney diseases improvement in comparison with losartan (ARB) post-irradiation.

Renin-angiotensin system (RAS):

RAS is a complex endocrine, paracrine, and autocrine system that modulates the function of the kidney (Nistala *et al*., 2009) which is activated in response to hypotension, decreased sodium concentration in the distal tubule, decreased blood volume and renal sympathetic nerve stimulation (Silverthorn, 2004 and Nistala *et al*., 2009).

Beside the importance of the RAS on the circulation and other organs, the local RAS in the kidney has attracted a great attention of research in last decades. The renal RAS plays an important role in the body fluid homeostasis. All major components and key enzymes for the establishment of a local RAS have been confirmed in the kidney. In addition to renal contribution to the systemic RAS, the intrarenal RAS plays a critical role in the regulation of renal function as well as in the development of kidney disease (Xu *et al*., 2009).



Coated from Irani and Xia (2008)

This system is classically known as a regulator of arterial pressure, which is accomplished by regulating the balance of water and sodium by their retention in the kidney leading to generating pressure. This has led to the successful development of anti-hypertensive drugs that block the system. In addition, this system has a fundamental role in the mechanisms of inflammation and self-defense for the cells and tissues of organisms.

Ang II regulates the synthesis of pro-inflammatory substances, since inflammation is the most basic mechanism found in all living organisms, allowing them to defend themselves against any aggressors. Renin was first discovered near the end of the 19th century as a pressor compound in the renal parenchyma of the rabbit. The common factor linking renal disease and cardiac hypertrophy is the increase of arterial pressure that results from the kidney's liberation of a vasoactive substance, which produces contraction of the vessels. Renin is an enzyme secreted by juxtaglomerular cells in the kidney that acts on angiotensinogen, a substance produced by the liver. When renin comes in contact with angiotensinogen, it cleaves the angiotensinogen molecule to yield a polypeptide named Ang I. As Ang I circulates in the blood, it flows through the lungs where a second enzyme called ACE a dipeptidyl carboxypeptidase converts Ang I to octapeptide Ang II by removing a single polypeptide. Ang II is active within the kidney. Ang II's primary effect is the retention of water and sodium. In the arterial system, Ang II is a peptide hormone that is responsible for a number of cellular events including vasoconstriction of vascular smooth muscle, aldosterone secretion from adrenal glomerulosa cells and trophic responses in tissue via binding with the AT1 (Ellis and Patterson, 1996; de Gasparo *et*

al., 2000 and Serrano *et al*., 2009). It has been demonstrated that the kidneys produce/express all of the RAS components (Nguyen, 2007).

One of the evolutionary goals of the development of the RAS is to protect the body by preserving salinity (Harris, 1983). However, it has become clear that an activated RAS can provoke detrimental effects as well. Pharmacological blockade of the RAS has significantly improved prognosis of patients with cardiovascular disease (Dahlof *et al*., 2002).

Interruption of the renin-angiotensin axis with a benazepril (ACEI) or losartan (ARB) slows the progression of chronic renal insufficiency in the presence or absence of diabetes (Hou *et al*., 2006). The use of ACEI and ARB was associated with a significant improvement in renal outcome (Hou *et al*., 2007).

Inhibitors of the RAS include ACEI, ARB, and aldosterone antagonists for the treatment of hypertension particularly where kidney disease is involved. The RAS plays an important role in the control of blood pressure and the pathogenesis of hypertension (de Gasparo *et al*., 2000). The biological actions of Ang II, the effector peptide for this system, are mediated by two predominant receptor subtypes, AT1 and AT2 (Carey *et al*., 2000 and de Gasparo *et al*., 2000). The AT1 receptor mediates virtually all of the known effects of Ang II that contribute to pathology in hypertension (Carey *et al*., 2000 and de Gasparo *et al*., 2000).

Ang II and bradykinin are important peptides involved in the regulation of vascular tone. Ang II is a vasoconstrictor and growth-promoting substance, whereas bradykinin is a potent vasodilator and growth inhibitor. ACE inhibitors, which are now widely used for the treatment of hypertension and heart failure, not only block the generation of Ang II from Ang I, but also prevent the degradation of bradykinin. Although originally it was thought that their beneficial effects were mainly due to blockade of Ang II generation, recent evidence suggests that bradykinin accumulation may be of equal importance (Farquharson and Struthers, 2002).

Ang II promotes the inflammatory response by the way of oxidative stress which is a biological mechanism that causes the physical concentrations of reactive oxygen species (ROS) to increase in the plasma, tissue, interior of the cells, and mostly in the mitochondria (de Cavanagh *et al*., 2007). It induces protein gene expression, which is necessary for the production and regulation of inflammation (Ferder *et al*., 2006).

Ang II inhibition produces changes in the mechanisms of oxidative stress, especially at the mitochondrial level. These effects may protect mitochondrial DNA from deletion, prevent increases in mitochondrial damage, and/or prevent decreases in mitochondrial numbers, all of which might be involved in the delay of normal aging processes (Ferder *et al*., 2002). Previous studies have shown that the RAS is pharmacologically blocked in normotensive rats. This resulted in biological changes in the aging mechanisms of different organs as well as producing an anti-inflammatory effect, which, both taken together brought about a

prolongation of life in the investigated animals (Vaziri *et al*., 2007, de Cavanagh *et al*., 2008 and Mueller and Nickenig, 2008).

The physiological roles of Ang II including regulation of the sympathetic system, fluid and water balance, hormonal control and vasoconstriction leading to increased blood pressure, are performed primarily via the AT1 receptor (McKinley *et al*., 2003). Antagonism of the influence of Ang II with ACE inhibitors (ACEI), such as perindopril, zofenopril and captopril, (Ambrosioni, 2007) and AT1 receptor antagonists as losartan and candesartan (Cheung, 2006) became widely used therapeutically as major targets for hypertension, cardiovascular disease and kidney disease. In addition to the therapeutic benefits of the use of ACE inhibitors and AT1 receptor antagonists in the treatment of hypertension, congestive heart failure and vascular disease, there are reports referring that these compounds may influence quality of life measures and cognitive functioning in both humans and animals (Kehoe and Wilcock, 2007).

ACEI and ARBs are capable of interfering with the activity of the RAS. At first sight, the modes of action of both ACEI and ARBs seem very similar, but after closer examination several differences are revealed.

Angiotensin converting enzyme and its inhibitors (ACEI):

ACE a di-peptidylcarboxypeptidase is expressed in endothelial, epithelial and neuroepithelial cells. It is composed of two domains, known as N- and C-domains, and it is primarily involved in blood pressure and it is an attractive target for drug

design due to its critical role in cardiovascular and renal diseases (Fernandez *et al*., 2004). The ACE (also known as kininase II) exists in the entire human body in both free and membrane-bound forms (Brown and Vaughan, 1998).

Somatic angiotensin converting enzyme (sACE) is composed of two domains, known as N- and C-domains (N-sACE and C-sACE). A testicular angiotensin converting enzyme (tACE) contains a single domain that shows high sequence identity to the C-terminal domain of sACE. Both domains have dipeptidyl-carboxypeptidase activity (Liu *et al*., 2001). Despite the high degree of sequence identity between N- and C-domains, they differ in substrate/inhibitor specificity (Cotton *et al*., 2002 and Hayashi *et al*., 2003). The N-domain is specific for the degradation of the tetrapeptide that controls hematopoietic stem cell proliferation and differentiation (Rousseau *et al*., 1995), whereas the C-domain is primarily involved in blood pressure regulation through the degradation of Ang I; a potent vasoconstrictor (Jaspard *et al*., 1993) and the inactivation of vasodilator peptide bradykinin (Villard and Soubrier, 1996).

ACE belongs to the class of zinc proteases that needs zinc and chloride for its activity. ACE plays an important physiological role in regulating blood pressure. ACE acts as an exopeptidase that cleaves dipeptides from the C-terminus of various oligopeptides. It converts an inactive form of a decapeptide Ang I to the potent vasoconstrictor octapeptide Ang II and inactivates the catalytic function of bradykinin (Richard *et al*., 2004). Specific inhibitors of ACE have been shown to be useful as antihypertensive drugs. Many synthetic ACE inhibitors including

captopril, enalapril, lisinopril and others are avaiable for clinical use (Brown and Vaughan, 1998). ACEI are well tolerated by most patients however some undesirable side effects may occur such as cough, lost of taste, renal impairment and angio-edema (Antonios and MacGregor, 1995). Jorde *et al*. (2000) demonstrated that even maximally recommended doses of ACEI could not completely prevent ACE-mediated Ang II formation.

With the discovery of ACE, an immediate search began for a method to inhibit its action (Cushman and Ondetti, 1991 and Erdos, 2006). Ferreira (1965) detected a potentiating factor for bradykinin that is derived from the poison of the *Bothrops jararaca*, a pitviper found in parts of Brazil, Paraguay and Argentina (Cushman and Ondetti, 1991). It was demonstrated that this derivative inhibits the ACE also (Cushman and Ondetti, 1991). Today, these ACEI, produced and marketed under a variety of names are among the top three most widely sold drugs in the world (Serrano *et al*., 2009). Another important effect of ACEI is their influence on the breakdown of bradykinin into inactive peptide (Coates, 2003). Bradykinin induces vasodilation by stimulating the formation of nitrogen oxide and metabolites of arachidonic acid in vascular endothelium (Vanhoutte, 1989). Furthermore, ACEI lead to elevated angiotensin 1–7 levels. The metabolite of Ang I and Ang II can stimulate the synthesis and excretion of vasodilatory prostaglandins, fortify the metabolic effects of bradykinin, and increase nitric oxide production (Tom *et al*., 2003). ACEI, such as captopril, are used to control hypertension. In patients and animals with primary nephropathies, these agents improve renal function more than that would be expected from their control of hypertension. *In vivo* or *in vitro*