

1. INTRODUCTION

The fact that angiotensin-converting enzyme inhibitors (ACEI) prevent not only the generation of angiotensin II (Ang II) but also the breakdown of kinins has given rise to the hypothesis that an increase in cardiac kinins could be a mechanism to protect the heart during hypertension, acute myocardial infarction or cardiac failure (for review see: Bhoola et al. 1992; Linz et al., 1995). In addition, it has been suggested that ACEI may increase the affinity of the bradykinin (BK) B2 receptor for its ligands, an alternative mechanism by which ACEI could potentiate the effects of kinins (Hecker et al., 1996). BK is the best investigated vasoactive effector peptide of the kallikrein-kinin system. It is cleaved from kininogen precursors by kininogenases such as kallikrein. Administration of BK to humans and several animal species engenders a variety of effects, including blood pressure reduction through endothelium-dependent vasorelaxation, changes in renal blood flow and tubular function, inflammatory reactions, vasoconstriction via direct effects on smooth muscle cells, alteration in vascular permeability and activation of sensory pain fibres (Armstrong et al., 1954; de Freitas et al., 1964; Willis et al., 1969; Levinsky et al., 1979). However, circulating concentrations of BK are usually low (Scicli et al., 1982), thus autocrine and paracrine actions of kinins have increasingly received scientific attention. Kinins are rapidly degraded by kininases, including kininase II, which is identical to angiotensin-converting enzyme (ACE). Inhibitors of ACE exert numerous actions on the cardiovascular system, some of which have been attributed to kinin potentiation. These actions include the prevention of neointima formation after endothelial denudation in rats (Farhy et al., 1993), preservation of endothelial function in experimental atherosclerosis in rabbits (Becker, et al 1991), reduction of postischemic reperfusion arrhythmias in isolated working rat hearts (Schölkens et al., 1988) and of infarct size in dogs (Ertl et al., 1982) cats (Lefer et al., 1984) and rats (Stauss et al., 1993; Duncan et al., 1996), or prevention of development of left ventricular hypertrophy (LVH) in rats with aortic banding (Linz et al., 1992) and improvement of cardiac function in spontaneously hypertensive rats (SHR) (Gohlke et al., 1994). Thus, kinin potentiation is believed to be involved in the therapeutic actions of ACEI, especially in ACEI-mediated cardioprotection.

1. 1 The kallikrein kinin system

Knowledge concerning the molecular elements of the kallikrein-kinin system is being constantly refined. Kinins are a group of biologically active peptides that involved in inflammation, smooth muscle contraction and pain processes. Thus far, several kinin peptides have been identified in mammalian species, such as the nonapeptide bradykinin (BK), the decapeptide Lysylbradykinin (kallidin), the undecapeptides Met-Lys-bradykinin, Ile-Ser-bradykinin (T-kinin), Met-T-kinin, and T-kinin-Leu. Among them, BK, kallidin and T-kinin are the best investigated. All kinins share the nonapeptide sequence of BK (-Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH) at the carboxy terminus and differ in size and amino acid structure. Kinins are produced by the cleavage of precursor molecules termed as kininogens. Three types of kininogen have been classified according to their molecular weight and susceptibility to the various kininogenases. Two of them, high- and low-molecular weight kininogen (HMWK and LMWK respectively), are present throughout the mammalian lineage, they are synthesised by the liver and circulating in the plasma; whereas the third type, T-kininogen seems to be unique for the rat (Bhoola et al., 1992). Enzymes that cleave kininogens and liberate kinins are kallikreins (kininogenases). Kallikreins are expressed in the liver (plasma kallikrein), or in exocrine glands (tissues or glandular kallikrein). Tissue kallikreins are widely distributed in organs, including heart, kidney, blood vessels, central nervous system, and intestine. Human tissue kallikrein preferentially release the kallidin from kininogens (Nacleiro et al., 1988). In the rat, tissue kallikrein also release BK, from the low- and high- molecular weight kininogens.

Three different pathways may lead to kinin formation during inflammation: (1) the generation of bradykinin as a result of activation of the Hageman factor (coagulation factor XII) and the production of plasma kallikrein; (2) the production of kallidin by tissue kallikreins; and (3) the action of cellular proteases in kinin formation. In plasma, the kallikrein precursor prekallikrein is activated by the coagulation cascade and release BK mainly from HMWK. In brief, HMWK circulate in plasma as a 1:1 stoichiometric complex. This complex, together with the Hageman factor, binds to negatively charged surface of collagen, once they are exposed by tissue damage, the Hageman factor is activated, prekallikrein is converted to kallikrein, and HMWK

itself is digested to release the nine amino acid peptide BK. In addition, kallikreins also act directly on the complement pathway with direct cleavage of the chemotactically active peptide C5a from the complement component C5. Cleavage of fibrinogen by plasmin results in a number of products including fibrinopeptide B, which potentiates the action of bradykinin and has also chemotactic activity for phagocytic cells.

Under normal conditions, circulating levels of kinin peptides are very low, usually less than 3 fmol/ml, and exhibit a short plasma 'half-life' of 15-30 seconds, kinins are rapidly inactivated by a group of peptidases termed as kininases. These kininases include: kininase I, neutral endopeptidases 24.11 (NEP, enkephalinase), aminopeptidase P (APP), and kininase II (identical to ACE), they differ in sites of action and locations. Among them, kininase II is the most efficient and the major mechanism for BK inactivation (for review see McLean et al., 2000). It removes the C-terminal dipeptide from BK or Lys-BK, and leads to their complete inactivation. In assays for BK-like peptides, it was shown that kininase II accounts for 40-70% of the hydrolysis of exogenous synthetic BK by serum or cardiac membrane preparations of humans, rabbits or rats (Decarie et al., 1996; Blais et al., 1997). Kininase II is located in high amounts at the luminal surface of endothelia cells, a prime location for not only the degrading of circulating BK but also the generation of the potent vasoconstrictor peptide Ang II. In contrast to kininase II, Kininase I enzymes play a lesser role in the deactivation of BK, being responsible for less than 10% of total BK metabolism. However, kininase I activity is of particular importance since it is believed to generate the des-Arg⁹-BK and Lys-des-Arg¹⁰-kallidin, the potent agonists for bradykinin B1 receptor (see below), from BK and Lys-BK respectively. Under normal conditions, these des-Arg⁹ metabolites are largely inactive, while in certain immuno-pathological situations, they produce responses characteristic of inflammation including vasodilation, odema, and leukocyte recruitment within the microcirculation (Gohlke et al., 1994).

1. 1. 1 Bradykinin B1 and B2 receptors

Through activation of specific cell surface receptors, kinins exert their biological effects. These effects are wide reaching, including acute pro-inflammatory effects such as smooth muscle contraction, induction of pain, vascular hyperpermeability, vasodilation, which are

related with increased release of prostaglandins and nitric oxide (NO). As potent vasoactive agents in the microcirculation, Kinins' inflammatory roles were extensively characterised, kinins have actions on the microvessel smooth muscle or endothelial cells promoting arteriolar vasodilation and plasma extravasation following inflammatory insult or tissue damage (Hall et al., 1992). Additionally, the antioxidant, antiarrhythmic effects and the anti-proliferative effects, such as suppressed fibroblast growth and collagenogenic activity (Gohlke et al., 1994).

At present, at least two types of cell surface receptors, termed bradykinin B1 receptor (B1R) and bradykinin B2 receptor (B2R) have been completely defined in many species such as rat, rabbit, guinea-pig, dog, mouse as well as human et al. (Marceau et al., 1995). The receptors have the characteristic membrane-spanning regions linked by three extra-cellular and four intracellular loop regions, they are linked to specific G-protein-coupled second messengers. Between the two subtypes, 36% of identical amino acid sequence has been demonstrated, and most of the homology is located in the seven transmembrane regions (Menke et al., 1994). This low homology between the two receptor subtypes suggests they may belong to very different regulatory mechanisms. Their agonists potency orders are different: the nonapeptide BK and the decapeptide kallidin, released from LMWK and HMWK by kallikrein in the blood and tissues, are the natural agonists for B2R; while both BK and kallidin are inactive for B1R. Degradation of kinins by an arginine-carboxy-peptidase with kininase I activity produces B1R agonists, the des-Arg⁹-BK and Lys-des-Arg¹⁰-kallidin. Under physiological conditions, B2R is present in many cell lines and tissues, such as the intestinal, cardiovascular, genitourinary and respiratory tracts and in ocular and neuronal tissues, where it mediates majority of the characterised actions of kinins, including effects such as modulation of renal blood flow, hypotension, cellular growth, bronchoconstriction and acute inflammation responses (Haasemann et al., 1994; Bhoola et al., 1992). In contrast, the B1R is normally absent, but following an inflammatory insult, its expression can be upregulated to a high level in a variety of cell types including vascular smooth muscle cells (SMC), endothelial cells (EC) and fibroblasts (FB) (McLean et al., 2000). Additionally, B1R and B2R differ significantly in regard to receptor-mediated ligand internalization: kinin agonists promote the B1R upregulation rather than receptor desensitisation and internalisation, which occurs with the B2R subtype, exposure of cloned human B2R to BK results in a rapid receptor mediated ligand internalisation and the sequestration of the receptor and the G protein α unit in

caveolae, accompanied by a profound loss of surface receptor binding (Austin et al., 1997; de Weerd and Leeb-Lundburg, 1997). This differential susceptibility to desensitisation leads to the hypothesis that during tissues injury or inflammation, an integrated system develops from B2R mediated acute inflammation to a sustained B1R mediated chronic, or at least prolonged, inflammatory response (for review see Couture et al., 2001).

A third bradykinin receptor, B3R, may exist in the microvasculature of the guinea pig hindbrain, and in cultured guinea pig trachea smooth muscle cells (Pyne et al., 1993; Regoli et al., 1993). However, definite proof still awaits further cloning efforts and the development of new receptor antagonists.

Receptor antagonists are inevitable tools for the study of receptor-mediated functions of the kallikrein-kinin system in both physiologic and pathophysiologic conditions, their developments are also crucial for define kinin receptor subtypes. In 1985, Vavrek and Stewart developed the B2R antagonist through substitution of D-phenylalanine for proline at position 7 of BK. Soonafter, HOE 140 (icatibant; D-Arg[Hyp³, Thi⁵, D-Tic⁷-Oic⁸]-BK) was discovered, it was termed a “representative of the second generation of B2R antagonists” for giving the most potent, stable and long-lasting duration of action in animal studies. HOE 140 binds tightly to the B2R (but not B1R) with a k_D of less than 0.05 nM, thereby outstripping the k_D of the natural ligand, BK (Menke et al., 1994). In the past years, metabolically stable B1R and mixed B1R + B2R antagonists of high affinity and stability have been synthesised. Such as B9858, which has some selectivity for B1Rs, and B9224 (Aca-D-Arg-[Hyp⁵, Igl⁵, D-Ig¹⁷, Oic⁸]-BK), containing the novel amino acid alpha-(2-indanyl)glycine and having extremely high potency at both B1R and B2R, and nonpeptidic BK antagonists have been developed (Stewart et al., 1996).

In the heart, the existence of an independent cardiac kallikrein-kinins system has been proven by growing evidences. Kininogen, kallikrein and B1R and B2R receptor mRNAs were detected in the rat ventricular myocardium as well as in cultured cardiomyocytes (CMC) (Yayama et al., 2000). Tissue kallikrein, its mRNA, and kininogen were detected in rat atria and ventricles (Nolly et al., 1994; Yoshida et al., 2000). Coronary arteries were also found contain and release kallikrein. Additionally, cardiac kinin levels have been reported much

higher than that in blood (Campbell et al., 1993). These evidences suggest the involvement of the local kallikrein-kinins system in the regulation of cardiac function.

1. 2 Kinins as antihypertensive and cardioprotective peptides

Various findings from both human and animal studies suggest that kinins may be involved in the beneficial effects of ACEI therapy. These evidences however, are sometimes conflicting or incomplete, and dependent on the experimental methods. In patients with hypertension, treatment of ACEI captopril effectively lowered the mean, systolic pressures, while the co-administration of icatibant, the B2R antagonist, significantly attenuated the acute hypotensive effect of captopril, and this effect was also observed in normotensive volunteers (Gainer et al., 1998). In the study of anaesthetized myocardial infarction (MI) dogs, it was shown that ACEI treatment and subsequent increase of BK level lead to beneficial cardiac effects, including reduction of the incidence as well as duration of ventricular fibrillation and an improvement of cardiodynamics via increased left ventricular pressure, contractility, and coronary flow, without changes in heart rate; While all of these beneficial effects were abolished in a concentration manner by perfusion with the B2R antagonist, icatibant, and the NOS inhibitor, L-NNA (Linz et al., 1992). In a recent study of patients with heart failure, it was observed that BK contributed to the vasodilation associated with chronic ACEI therapy, and this effect was through the activation of B1 receptor (Witherow et al., 2001). Notably, in hypertension rat models, it seems that endogenous kinins are involved in the blood pressure-lowering effects of ACEIs only in the renin-dependent hypertension: in the two-kidney, one clip chronically hypertensive rats, the acute blood pressure-lowering effect of the ACEI captopril was dramatically blocked by pretreatment with anti-kinin bodies, while this effect could not be seen in sodium-depleted normotensive rats (Carretero et al., 1981).

In fact, the kallikrein kinin system has been associated with cardiovascular regulation since early of their discovery. Several decades ago, Eliot and colleagues demonstrated that intravenously injection of urinary kallikrein elicited a profound decrease in blood pressure. After that, both in vivo and in vitro studies suggested that BK was a hypotensive agent; BK or des arg-BK could increase local blood flow as a vasodilator and lead to decreased blood

pressure (Carretero et al., 1981; Lamontangen et al., 1996). Studies in a mouse strain with targeted disruption of the B2R gene showed that mice lacking a functional B2R gene (B2R^{-/-}) had higher blood pressures and heavier hearts than wild type mice, as well as exaggerated pressure responses to exogenous Ang II and to chronic dietary salt supplementation (Madeddu et al., 1997). In transgenic mice, over-expressed human tissue kallikrein induced hypotension, and administration of aprotinin, a potent tissue kallikrein inhibitor, restored the blood pressure of the transgenic mice but had no significant effect on control littermates (Wang et al., 1994). These antihypertensive effects of kinins were thought through an endothelial-dependent mechanism related to the release of prostaglandins and nitric oxide (NO) that produce reductions in vascular resistance. It is believed that in the vasculature, BK-mediated activation of endothelial B2R may stimulate endogenous nitric oxide synthase (NOS), thus increasing NO and counteracting the effects of Ang II by inhibiting contraction and growth of smooth muscle cells (Carretero et al., 1981). However, the cardiovascular response elicited by BK may differ via various mechanisms and exert complex effects, some investigations suggested that via neural mechanisms, BK may also elicit hypertensive effects. As a potent stimulator of sensory afferent neurons, BK can activate sympathoexcitatory reflex mechanisms by increasing afferent inputs to central cardiovascular and renal integration centres (Walker et al., 1995).

In addition to their vasoactive effect, endogenous kinins (mainly BK) may also play important roles in the anti-ischemia conditions during heart diseases. In the process of ischemic heart diseases such like MI, the ischemic myocardium undergoes a phase termed as preconditioning, short periods of ischemia stress results in a marked, albeit temporary, resistance of the myocardium to a subsequent, more prolonged period of that same stress, thus reduce ischemic cellular damage and life-threatening ventricular arrhythmias (Murry et al., 1986; Remme 1997). Evidences for a role of kinins in the powerful cardioprotective mechanism were obtained from both animal models and human studies. In a dog MI model, there was a marked increase BK levels in coronary sinus in preconditioned dogs before the prolonged coronary artery occlusion (637 ± 293 pg/ml compared with 114 ± 41 pg/ml in nonpreconditioned dogs), and this preconditioning procedure markedly reduces ischemia-induced ventricular arrhythmias (Parratt et al., 1997). In a recent study, it was documented that the B2R was involved in the late phase of preconditioning in rabbit heart, ischemic preconditioning or/and

BK caused decrease in infarct size while HOE140 blocked the infarct limitation (Kospitprapa et al., 2001). In another gene delivery study, after kallikrein gene delivery, cardiac kinin and cGMP levels were significantly elevated, and cardiac-protective effects such as attenuated myocardial infarction, ventricular arrhythmias, and apoptosis in ischemia/reperfusion injury via kinin-cGMP signal pathway were confirmed compared with the control (Yoshida et al., 2000). The concept that BK may play cardioprotective roles during ischemic preconditioning was also supported by clinical observation, Leesar and coworkers demonstrated that intracoronary infusion of BK before percutaneous transluminal coronary angioplasty significantly attenuated the severity of the ischemic injury of the patients (Leesar et al., 1999).

During MI, kinins are released directly from the myocardium, which can be maintained for several weeks, increased plasma levels of kallikrein and kininogen were also demonstrated (Baumgarten et al., 1993;). It was thought that the increased release of cardiac BK during MI may offset the increase in myocardial oxygen demand imposed by enhanced local production of catecholamines and Ang II. However, there is controversy regarding the protective effects of BK in myocardial ischemia. Duncan et al. (1996) did not find a significant increase in cardiac tissue BK levels during ACEI treatment started 48 hours after induction of MI. Seyedi and coworkers demonstrated that an active kallikrein-kinin system in a preparation of sympathetic nerve endings from the guinea pig heart produced norepinephrine exocytosis when BK synthesis was increased or when its breakdown was retarded by ACEI treatment. These findings raise the possibility of a differential effect of BK accumulation in the cardiac interstitium, where it interacts directly with nerve terminals and fibroblasts and may have a deleterious effect by promoting norepinephrine release and perivascular/myocardial fibrosis (Seyedi et al., 1997). Further study is needed to test this intriguing hypothesis.

1. 3 Regulation of B1R and B2R after induction of myocardial infarction

The existence of two bradykinin B1 and B2 receptor was described by Regoli and colleagues in the late 1970s, however, it was only within the last few years that these two subtypes has been definitely confirmed, and despite the well documented roles of the kallikrein-kinin system involved in the cardiovascular diseases, studies regarding the behaviour of kinin

receptors under such pathophysiological conditions are lacking, many works are mainly focused on their contribution to the proinflammatory roles of kinins. It has been known that acute MI can be assessed by activation of the kallikrein-kinin system and several BK-mediated effects can be abolished by B2R antagonists. To further elucidate the behaviour of both kinin receptors under this condition, our research group investigated the expression of both B1 and B2 receptors in the heart after MI induction (Tschöpe et al., 2000 *a, b*). Rats were submitted to permanent occlusion of the left descending coronary artery. 6 h, 24 h, or 6 days after MI induction, the left ventricle (LV), right ventricle (RV) were isolated and both B1R and B2R mRNA expression were analysed by an RNase-protection assay technique, western blot analysis was used to determine protein levels of the receptors in the infarcted area of the LV. Results showed that basic expression of B2R was detectable in the LV and RV of the heart in non-operated rats; An up-regulation of B2R expression occurred as early as 6 h after MI induction, it reached to a peak at 24 h and high level of B2R mRNA was maintained in the LV for at least 6 days. This up-regulation correlated with an increase in the protein level of the B2R in the scar of the LV at least 6 days after MI induction. In contrast, no basic B1R expression was detected. Its expression was firstly detected 6 h after MI induction, reached its maximum 24 h after coronary ligation, and then significantly reduced to a level comparable to that measured 6 h after MI, showing a different time curve compared with B2R (Fig 1.2). These findings suggested that the known increase in BK levels after induction of MI is accompanied by B1R and B2R up-regulation in the early stages of cardiac wound healing.

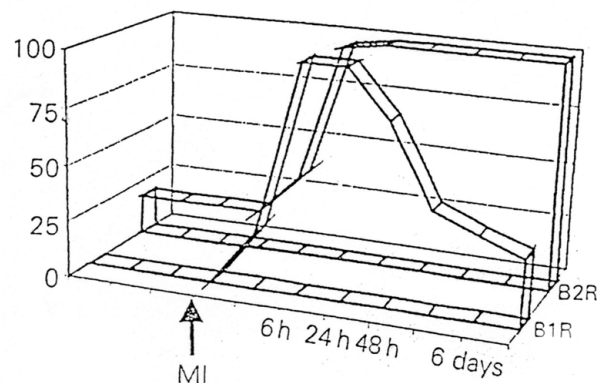


Fig 1.2 Time curve for the expression of the kinin receptors. B1R and B2R in the left ventricle 6 h, 24 h, 48 h and 6 days after induction of MI. In both cases, the number for the highest expression was taken as 100% (from Tschöpe et al., 2000 *a*).

The mechanism of the upregulation of both kinin receptors after MI induction, however, is still unclear. As mentioned above, in both *in vivo* and *in vitro* models B2R showed a rapid desensitization after chronic stimulation, thus, it would be expected that the kinin-related cardioprotective effects of increased release of BK might be limited by a down-regulation of B2R under these conditions. However, both B1R and B2R were upregulated in the LV, suggesting that early after MI, the heart may initiate certain mechanisms which will enable a sustained release of B2R, thus maintain the kinin-related cardioprotective effects. Possible mechanisms for upregulation of both kinin receptors however, may include the also high released cytokines, like interleukin 1 β (IL1 β), which is thought to be the optimal inducer of B1R in various cell types, through transcription as well as post-transcriptional mRNA stabilisation (Zhou et al., 1998; Ni et al., 1998; McLean et al., 1999). Thus, it is reasonable that cytokines may force kinin-dependent vasodilation and vascular hyperpermeability by BK receptor induction, both important for initiating subsequent repair phase.

1.4 Cytokine family

Early after MI, the heart overexpresses a class of biologically active factors, which are generally referred as cytokines. Cytokines are a group of relatively small molecular weight protein molecules, they are secreted by various cells types in response to a variety of different inducing stimuli (Fig 1.3). To date approximately 100 molecules have been cloned, they act as humoral regulators at nano- to picomolar concentrations, modulate the functional activities of individual cells and tissues physiologically and/or pathophysiologically, involved in virtually all general systemic reactions of an organism, including such important processes as wound healing, inflammatory processes, and the regulation of immune responses. In terms of cardiac pathophysiology, the cytokines may be divided into three categories: proinflammatory, anti-proinflammatory, and cardioprotective. They may originate from inflammatory cells: polymorphonuclear cells, monocytes, lymphocytes, or mast cells; and also from resident cells: fibroblasts, endothelial cells, other cells or even cardiomyocytes themselves. The biological activities of cytokines are mediated by specific membrane receptors, which can be expressed on virtually all cell types known (Pulkki et al., 1997).

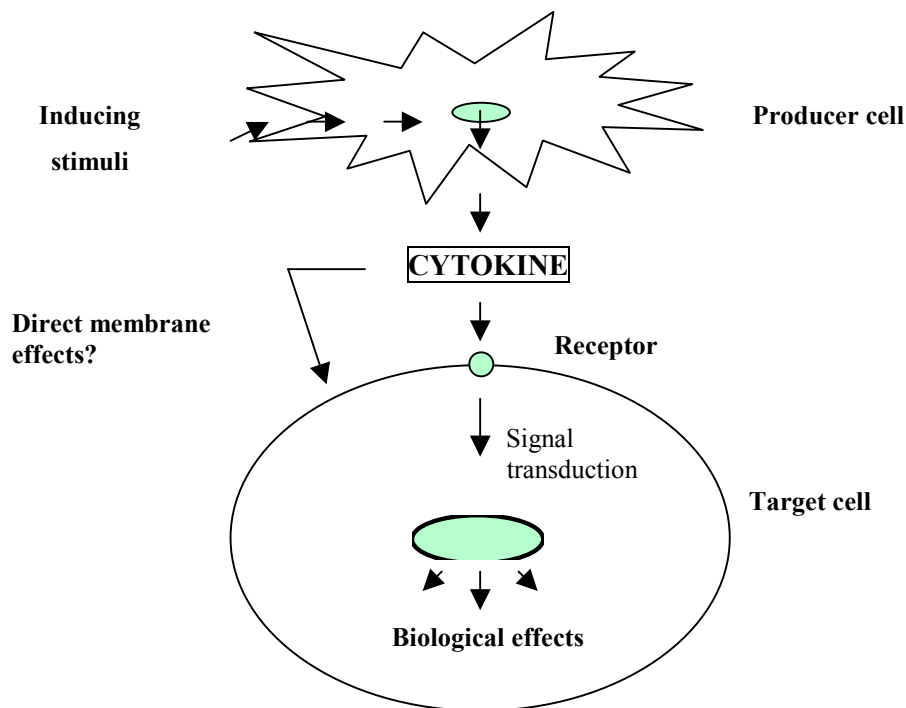


Fig 1.3 Mechanisms of cytokines action. Cytokine secreted by "producer cells" in response to a stimulus act on "target cells". While cytokines are thought to exert their effects by binding to specific receptors on the surface of the cell, they may in some instances have direct membrane effects. Activation of the specific cytokine receptor is then thought to lead to the induction of a variety of different biological effects through activation of one or more different intracellular signalling pathways, cytokines secreted by 'producer cells' influence the biologic behaviour of 'target cells' by both autocrine and paracrine fashion (Mann et al., 1994).

“Proinflammatory cytokines” are those cytokines that are responsible both for initiating the primary host response to a bacterial infection, as well as tissue repair after tissue injury. Under inflammatory conditions, this cellular immunity has an important role in the regulation of wound repair: blood vessels in the affected part expand and cause more blood to flow into the area. Leukocytes may leave the blood vessels and enter the surrounding tissue, proinflammatory cytokines were secreted, including tumour necrosis factor alpha (TNF α), interleukin 1 alpha, beta (IL1 α , IL1 β), interleukin 6 (IL6) and interleukin 8 (IL8). Each of these cytokines is also capable of influencing the expression of the other proinflammatory

cytokines. It has been revealed that these cytokines help to protect against infection, prepare injured tissue for repair, and enhance phagocyte recruitment and activation. Some authors reported that cytokines released by the recruited cells regulate the ability of fibroblasts and epithelial cells to remodel damaged tissue (Lowry et al., 1993; Barbul et al., 1990).

Cytokines can be produced by different cell types, for instance, although the major source of IL1 β is monocytes, almost every cell type in the body can produce IL1 β in response to a variety of stimuli, such as lipopolysaccharide (LPS), microbial toxins, products of activated lymphocytes, complement, components of the clotting system, as well as by IL1 itself.

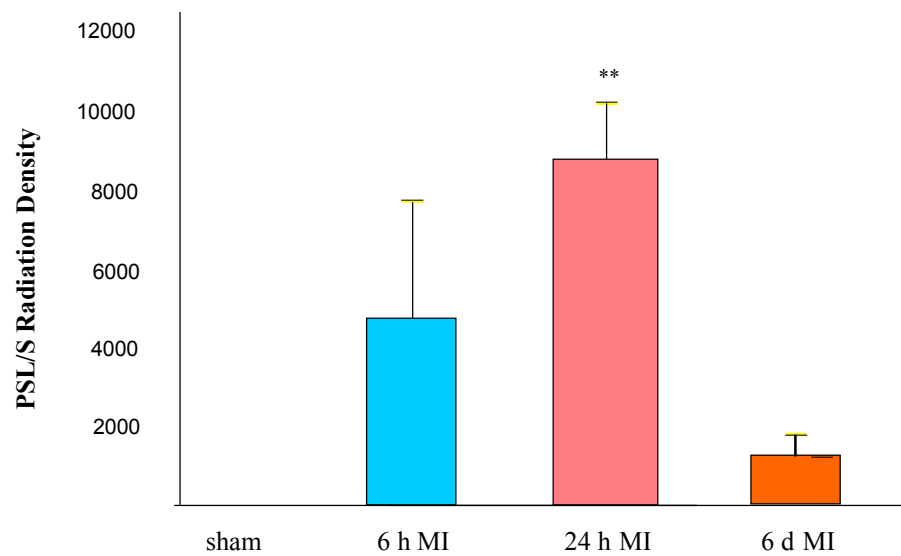
1.5 Cytokines and myocardial infarction

Large body of researches on cytokines have focused in the pathogenesis of sepsis and septic shock, however, their roles in the pathogenesis of acute ischemic event associated with MI, the progression of cardiomyopathies has been a relatively recent discovery. Their effects on immune-mediated myocardial function are studied in both human and animal models. Considerable attention has been devoted to a potential role for cytokines and growth factors that are known to be increased post-myocardial infarction. In patients early after experiencing acute MI, increased plasma levels as well as local myocardial production of IL1 β and several other proinflammatory cytokines, like IL6, IL8, and TNF α have been observed (Guillen et al., 1995; Neumann et al., 1995; Tashiro et al., 1995; Blum et al., 1994). In a recent study of rat model of myocardial infarction, increased release of TNF α , IL1 β and IL6 were documented in the infarcted region of the heart, in the noninfarcted region, the release of IL1 β was highest among these cytokines and maintained at a high level for at least 20 weeks (Ono et al., 1998). The cause for this cytokine overexpression is uncertain. However they may exert complex effects, evidence suggests that these proinflammatory cytokines are capable of modulating myocardial function through a variety of mechanisms, such as promoting left ventricular remodelling, inducing contractile dysfunction, and uncoupling myocardial β -adrenergic receptors. During MI, alterations in collagen concentration and phenotypes have been demonstrated in ischemic cardiomyopathy, and IL1 may regulate the production, release, and activation of metalloproteinases, which are important in the destruction and remodelling of the connective tissue matrix (McCormick et al., 1994). Okusawa and co-workers showed that

IL1 β , when given as a single bolus, led to a rapid decrease in systemic arterial pressure in rabbits within 60 min of injection (Okusawa et al., 1988). Hosenpud et al. (1989) extended these findings and showed that treatment with IL1 β depressed myocardial force generation in adult rat hearts studied in vitro. In the article by Balligand et al. (1993), IL1 had no effect on basal contractile function, but it depressed isoproterenol stimulated increase in contractility in neonatal and adult rat myocyte preparations, and the depression in IL1 induced contractility was shown to be through a NO signalling pathway. However, despite the well-documented deleterious effects of cytokines on the myocytes and myocardial function, an early increase in the release of cytokines in patients with MI seems to be essential for the healing process and recovering of the patients. In the article by Blum, clinical findings proved that patients with a grave outcome had lower IL1 level on the first day than patients with a good outcome, patients who could not raise IL1 to higher levels shortly after the myocardial damage had a bad outlook. The authors followed 39 patients who were admitted with acute myocardial infarction, IL1 β was measured in the first 24 h, day 4, day 7 and compared among different groups, it was thought that the immune system had some influence on the healing process, and patients who did not have the ability to respond efficiently enough on time had a worse outcome (Blum, 1996).

Early after MI, the heart experiences the exudative phase of wound healing (inflammatory phase). Under this condition, the activation of BK may correlate with the cytokine release. As a potent inflammatory mediator, BK stimulates the synthesis of cytokines IL1 β , IL2 and IL6 (Pagaelow et al., 1995; Pan et al., 1996). Migration of macrophages and the formation of fibroblasts may enhance the inflammatory effects of BK since these cell types are known to express BK receptors. Induction of additional cytokines may extend the proinflammatory function of BK to contribute inflammation and tissue repairing. IL1 β can induce B1R upregulation through an induction of transcriptional as well as post-transcriptional mRNA stabilisation (Zhou et al., 1998), some authors suggest that it can also induce B2R through a prostanoid cyclic AMP-dependent pathway (Schmidlin et al., 1998). The effects of cytokines on kinin receptors may differ with each other. For instance, in inflammation, despite the involvement of the closely related cytokine tumour necrosis factor- α (TNF- α), it was reported that this cytokine does not play a role in BK -B1 receptor mediated inflammatory hyperalgesia (Davis et al., 1994).

Using rat model of MI, our research group also investigated the expression of IL1 β in the left ventricle post MI. In agreement with previous works, induction of MI caused a dramatic upregulation of IL1 β in the ventricular myocardium. As shown in Fig 1. 4, IL1 β mRNA was not detected in time-matched sham operated rats, in the infarcted myocardium, it was firstly detected at 6 h after MI induction, reached its peak at 24 h, and then dropped to a lower level at 6 d after coronary occlusion. As mentioned above, the upregulation of IL1 β in noninfarcted area of the could be more prominent (Ono et al., 1998), indicating an involvement in the acute infarction pathogenesis.



*Fig 1. 4 Effects of myocardial infarction on proinflammatory cytokine IL1 β mRNA expression. Expression of IL1 β mRNA was induced at 6 h after MI and reached its peak at 24h but dropped to a lower level at 6 d after MI induction. ** $P < 0.01$ versus sham (from Tschöpe et al., unpublished data).*

1. 6 Objectives of research

Taken together, via activation of B1 and/or B2 receptors, kinins play important roles in mediating the pathogenesis of cardiovascular disease. After myocardial infarction, release of components of the kallikrein kinin system such as kallikrein, kininogen as well as kinins is increased. Recent study from rat myocardial infarction model revealed that the known increase of bradykinin early after MI is accompanied by an upregulation of both bradykinin B1 and B2 receptor expression. Cytokines, such like IL1 β , which is also overexpressed during MI, are capable of modulating the release of bradykinin and kinin receptor expression in many cell systems under inflammatory conditions. To get a further view of the co-relationship between cytokine IL1 β and the expression of kinin B1 and B2 receptors in vascular and cardiac cell systems, the present study was carried out 1), to characterize the basic expression of B1 and B2 receptor in cardiac myocytes, cardiac fibroblasts and aortic smooth muscle cells, 2), to describe the possible influence of IL1 β on the expression of both kinin receptors in these cell types. If so, 3), to investigate the time pattern of this regulatory effects; and 4), using a myocardial infarction rat model to characterize whether a blocked IL1 β production by an inhibition of IL1 β converting enzyme inhibitor (ICEI) affects the release of kinin receptors in the heart.