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Disease mechanisms and emergence therapies: protein kinases and their inhibitors in cardiovascular diseases*

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[ABSTRACT] Cardiovascular diseases (CVDs) are a major cause of morbidity and mortality in the world. So far, there has been substantial progress toward understanding the pathophysiology and treatment of CVDs. There are multiple cell signaling cascades, some of which are beneficial or compensatory and others deleterious. The balance between these pathways determines the outcome as a diseased or non - diseased state. Protein phosphorylation, which is mediated by enzymes, called protein kinases, is a major mechanism for transducing external stimuli into intracellular signals. Electively targeting of signaling pathways using protein kinase inhibitors would have a potential advantage over receptor blockers. By now, there are types of protein kinase inhibitiors available for treating several diseases. The success of kinase inhibitors in cancer treatment has strongly supported application in the treatment of CVDs. Here, we will review several kinds of protein kinases as potential targets for CVDs and some difficulty in identifying a protein kinase as a putative therapeutic target for CVDs.

[KEY WORDS] Protein kinases; Protein kinase inhibitors; Cardiovascular diseases

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Cardiovascular diseases (CVDs) are a major cause of morbidity and mortality in the world. So far, there has been substantial progress toward understanding the pathophysiology and treatment of cardiovascular diseases (CVDs)[1].

Protein phosphorylation reactions are mediated by enzymes, called protein kinases. Protein kinases are covalently modify proteins by attaching phosphate groups (from ATP) to serine, threonine, and/or tyrosine residues and they represent a large family with an essential role in cellular processes, particularly in signal transduction responses of cells to the external environment. Protein kinases regulate most aspects of normal cellular function. The human genome encodes about 500 protein kinases[2]. Two major types are tyrosine kinases, which number about 90 in the human genome and serine - threonine kinases, of which 388 have been identified. The tyrosine kinases can be further classified into receptor tyrosine kinases and non - receptor tyrosine kinases, both of which have been targeted for therapeutic potential.

The pathophysiological dysfunction of protein kinase signaling pathways underlies the molecular basis of many cancers and of several manifestations of cardiovascular disease, such as hypertrophy and other types of left ventricular remodeling, ischemia/reperfusion injury, angiogenesis, and atherogenesis[3]. Selective targeting of signaling pathways using protein kinase inhibitors, would have a potential advantage over receptor blockers[1].

In this review we focus on GPCR - activated protein kinases, with an emphasis on signaling activity in the cardio vascular system. In particular, the multifunctional calcium/ calmodulin - dependent protein kinase II (CaMKII) will be highlighted as a paradigm for understanding various approaches to developing serine/ threonine protein kinase inhibition, and because new evidence shows CaMKII to be a validated target in structural heart disease. Although protein kinases arrived late to the drug development table, they are now being targeted by more drug development programs than any other class of protein other than GPCRs.

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1 Cardiovascular disease linked to protein kinase dysregulation

Many protein kinases are implicated in cardiovascular disease (Tab 1). The long list of candidate molecules precludes a detailed discussion of each protein kinase that could be a therapeutic candidate, but we have selected several general points concerning the role of protein kinase in cardiovascular disease.

1.1 Dynamics and isoforms of protein kinase in the development of cardiovascular diseases

① The GRKs family G protein – coupled receptor kinases (GRKs) constitute a family of seven serine/threonine protein kinases that specifically recognize and phosphorylate agonist – activated G protein – coupled receptors (GPCRs). GRK – mediated receptor phosphorylation is one of the well – characterized mechanisms for GPCR desensitization. Receptor phosphorylation triggers the binding of arrestins, which block the activation of G proteins, leading to rapid homologous desensitization. As a result of β – arrestin binding, phosphorylated receptors are targeted for clathrin – mediated endocytosis, a process that classically serves to resensitize and recycle receptors back to the plasma membrane, but that has gained renewed interest since it can also help promote the activation of additional signaling pathways by way of arrestins acting as agonist – regulated adaptor scaffolds[4]. GRK family members can be subdivided into three main groups based on sequence homology: rhodopsin kinase or visual GRK subfamily (GRK1 and GRK7), the β – adrenergic receptor kinases subfamily (GRK2/GRK3) and the GRK4 subfamily (GRK4, GRK5 and GRK6). These kinases share certain characteristics but are distinct enzymes with specific regulatory properties.

② The CaMK family There are three types of CaMK: I, II, and IV. Type II (CaMKII) is the most abundant in heart, while type IV is absent or present in such low abundance as to be unlikely to play any significant role[5]. There are four isoforms of CaMKII, α, β, δ and γ, that are encoded by distinct genes, and each of these isoforms can exist as a variety of splice variants. Expression of the α and β isoforms is largely restricted to neuronal tissue, while the δ and γ isoforms are both present in diverse tissues including heart. The δ isoform is the prominent cardiac isoform, and the relative expression of different δ variants changes throughout cardiac development and disease.

③ The PKC isoforms PKC is involved in vasoconstriction, ischemic preconditioning, and cardiac hypertrophy. The PKC isoforms have a tissue – specific expression pattern. They are grouped based on their requirement of calcium and diacyl – glycerol (DAG). The classical PKCs (cPKCs; α, β_1, β_2 and γ) are activated by both calcium and DAG. The novel PKCs (nPKCs; δ, ε, η, μ and θ) are activated by DAG but not by calcium, and the atypical PKCs (aPKCs; ζ and ι) require neither DAG nor calcium. The role of several isoforms, PKC – α, PKC – β, PKC – δ and PKC – ε, is known in greater detail. PKC signaling pathways are activated by all GPCRs that couple to $G\alpha_q$ heterotrimeric G proteins. These include receptors for the neurohormonal ligands, Ang II, ET – 1, and phenylephrine, all with established cardiovascular effects.

④ Mitogen – activated protein kinase Mitogen – activated protein kinases (MAPKs), ERK1/2, JNKs, and p38MAPKs, are the terminal components of a three – tiered signaling cascade. These signaling cascades control complex programs, such as embryogenesis, differentiation, proliferation and cell death, in addition to short – term changes required for homeostasis and acute hormonal responses. Despite their ubiquitous nature, the mechanism of activation, substrates, and functions are unique and often cell specific. ERK1/2, the first of the MAPKs to be identified, are activated by growth stimuli and have growth and cytoprotective functions. These are activated by ET – 1, phenylephrine, and Ang II in cardiac myocytes. ERK1/2 are generally considered to potentiate the development of compensatory hypertrophy. On the other hand, JNKs and p38MAPKs are activated by cellular stress such as ischemia, pro – inflammatory cytokines, and hypertrophic stimuli.

⑤ Other protein kinase Such as glycogen synthase kinase (GSK) – 3β, Rho GTPases (ROCK) mammalian target of rapamycin (mTOR) will be illustrated in the following part of this review.

1.2 Location, cell type and species

The specificity of protein kinase signaling is refined by the pattern of subcellular localization. Many protein kinases have adapter proteins to direct their activity. Proper targeting of kinases to proteins is essential for normal function, and disruption of targeting by peptide or small – molecule inhibitors is a potential strategy for inhibiting protein kinase activity. Signaling networks can be cell – type specific. Studies in cultured cells,

including neonatal cardiomyocytes, are advantageous because these cells typically grow and divide in a stable manner, form intercellular connections, and are readily available. On the other hand, significant differences exist between cultured cells and fully differentiated adult cells. These differences can include protein kinase populations, ultra structural localization of protein kinases and their protein targets, and the relationships between GPCRs, protein kinases and the biological responses to GPCR and protein kinase activation (or inhibition). Neonatal cardiomyocytes form the main source of cells for studying protein kinase signaling, but the consequences of perturbing protein kinase signaling networks in neonatal cells might not recapitulate results *in vivo* or in fully differentiated adult cells[6]. While *in vitro* and *in vivo* approaches are important for studying the biology of protein kinases in the cardiovascular system, models using a combination of genetic and pharmacologic approaches for manipulating protein kinase activity are particularly appealing forvalidating the role of candidate protein kinases in disease mechanisms *in vivo*. By necessity, early *in vivo* validation studies are frequently performed in mice. Additional validation of murine findings in larger animal models is also important, however, given the significant differences in heart rate, metabolism, cellular calcium handling and action potential durations between mice and humans.

Tab 1 Protein kinases in diseased human hearts and in models of cardiovascular diseases

Kinase	Direction of alteration in expression or activity in diseased human tissue	Results of overexpression or increased activation	Results of knockout, inhibition, or mislocalization
PKA	Increased activity	Cardiac hypertrophy, dysfunction, and sudden death	ND
CaMKII	Increased expression	Cardiac hypertrophy, dysfunction, and sudden death, arrhythmias	Reduced remodeling and improved function after MI and isoproterenol KN62 and KN93 provided usefully in cardiac myocytes
PKC	Increased activity and expression	PKCβ improved function after ischemia, hypertension	Protection from ischemia by PKCε translocation
ERK	No change in activity in ischemic myopathy	ERK1/2 activity causes cardiac hypertrophy and increased contraction	ND
p38	MAPK activity increased in ischemic myopathy and after LVAD support	Fibrosis, dysfunction, and sudden death without hypertrophy	DN – p38 causes hypertrophy with reduced fibrosis during aortic banding increased hypertrophy with aortic banding, angiotensin II and isoproterenol
JNK	Activity increased in ischemic myopathyand decreased after LVAD support	Atrial enlargement	DN – JNK 1/2 increased hypertrophy with aortic banding and aging
JAK/STAT	Increased JAK1 and decreased JAK2 activation in DCM	Activation by GCSF promotes cell survival	JAK inhibition reduces ischemic preconditioning
c – AKT	Decreased activity after LVAD support	Activated c – AKT preserves function after ischemia reperfusion, but with increased MI size	DN – AKT reduced recovery after ischemia
GSK – 3β	Decreased activity and expression	Reduce myocardial cell death, following ischemia and reperfusion	Inhibition of GSK – 3β showed to reduce myocardial cell death, following ischemia and reperfusion
AMPK	Increased anabolic processes (lipogenesis) and enhance glucose uptake	AMPK had been linked to hypertrophic cardiomyopathy and to ventricular preexcitation	ND

CaMKII: multifunctional calcium/calmodulin – dependent protein kinase II; DCM: dilated cardiomyopathy; DN: dominant negative mutant; ERK: extracellular – signaling – related kinases; GCSF: granulocyte colony – stimulating factor; JAK: Janus kinases; JAK/STAT: Janus kinases/signal transducer and activator of transcription; JNK: jun N – terminal kinase; LVAD: left ventricular assist device; MI: myocardial infarction; ND: not described; p38 MAPK: a mitogen – activated protein kinase; PKA: protein kinase A; PKC: protein kinase C; AMPK: 5' – AMP – activated protein kinase; GSK – 3β: glycogen synthase kinase – 3β.

2 Strategies for identifying and designing kinase inhibitors

By now, there are there types of protein kinase inhibitions available for several diseases.

2.1 The first type is allosteric kinase inhibition through altered ATP binding including Gleevec, BIRB796, BAY43 – 9006 and AAL – 993. Gleevec is a potent allosteric inhibitor of several tyrosine kinases. These kinases include Bcr – Abl, platelet – derived growth factor receptor (PDGF – R) and stem cell factor receptor (c – Kit). Gleevec has been successfully used in the treatment of these cancers[7]. BAY43 – 9006 that binds B – Raf and AAL – 993 that binds the vascular endothelial growth factor – receptor (VEGF – R). Most small molecule protein kinase inhibitors interact with the conserved ATP – binding site of their target protein kinase. These ATP – competitive protein kinase inhibitors must ultimately target the kinase with high affinity to compete with the high intracellular concentrations of ATP, but sometimes they do not discriminate between the ATP – binding sites conserved in protein kinases and other ATP – binding proteins[8]. This lower specificity for their intended target may limit their clinical use, particularly when there are off – target side effects.

2.2 The second type is other ATP non – competitive allosteric inhibitors disrupt protein substrate interactions including PD09859 and other MEK inhibitors CMPD1, Akt inhibitors, glycogen synthase kinase – 3β inhibitors, BMS – 345541, GNF – 1 and GFN – 2. The most commonly studied MEK inhibitor has been PD098059. Kinetic analysis has subsequently revealed that PD098059 is non – competitive both with respect to ATP and their protein substrate ERK. PD098059 binds to a common or two overlapping sites on MEK. At the same time the structural basis for interaction with MEK remains to be determined, docking studies suggest that they bind to the same allosteric site of MEK as PD098059[9]. CMPD1 is an inhibitor of the p38 MAPK mediated phosphorylation of the downstream substrate MK2[10]. Additional experiments confirmed that CMPD1 interacted with p38 MAPK, rather than substrate, making CMPD1 an example for a small group of substrate selective protein kinase inhibitors[10] Akt (protein kinase B/PKB), a phosphatidylinositol (3,4,5) P3 binding protein kinase, plays a key role in the regulation of cell survival, proliferation and growth, and screening approaches have been used to identify selective inhibitors. Inhibition of both Akt1 and Akt2 appears to result in greater sensitisation of cancer cells to apoptotic stimuli[11]. In addition, ATP – non – competitive inhibitors of glycogen synthase kinase – 3β (GSK – 3β) have been described as the new drug leads for the treatment of Alzheimer's disease, diabetes, chronic inflammatory disorders and central nervous system disorders[12]. BMS – 345541 has been identified as an inhibitor of the IκB kinases IKK – 1 and IKK – 2. The therapeutic potential of IKK inhibition is emphasised by the efficacy of these inhibitors *in vivo* to inhibit lipopolysaccharide – induced TNF – α production, to reduce in joint destruction and inflammation in a collagen – induced arthritis model, to reduce inflammation in ischaemic brain damage and to induce apoptosis of melanoma cells[13,14]. Finally a new class of allosteric inhibitors of Bcr – abl has been recently reported[15], and GNF – 1 and GNF – 2 are examples and they bind to the myristoyl – binding pocket in the C – terminal lobe of Bcr – abl[15].

2.3 The third type is inhibitors that compete with protein substrates. The key recent examples are considered in this section, beginning with the targeting of the MAPKs. Other small – molecule inhibitors of protein kinases outside the MAPK family have also shown direct competitive inhibition with the protein substrate. For example, ON01910 is a substrate competitive inhibitor of polo – like kinase – 1. It is a new lead for cancer therapy in combination with, or instead of, ATP – competitive polo – like kinase – 1 inhibitors such as BI2536, or inhibitors such as scytonemin that are characterised kinetically as mixed inhibitors[16]. But whether the efficacy of ON01910 in inhibiting tumour growth is the direct result of inhibiting polo – like kinase – 1 or other protein kinases therefore remains an issue to be explored in greater detail. AG538 has been identified as a protein substrate competitive inhibitor of the insulin – like growth factor receptor 1 (IGF – R1) and has been further modified by replacing the catechol moiety with benzoxazolone groups on either side of the molecule to enhance its stability in cells.

3 Protein kinases potential targets for cardiovascular disease

As we know most of the above kinase inhibitors in development are for the treatment of cancer, though the nature of involvement of kinases in cancer is different than in CVDs. In cancer, the aberrant kinase activity is an intrinsic property due to mutations, genetic or somatic, while in CVDs this is due to enhanced stimulation by activated neuro – hormonal systems[17]. The success of kinase inhibitors in cancer treatment has strongly supported application in the treatment of CVDs. Two kinds of kinase inhibitors are being developed, antibodies and small molecule kinase inhibitors. The antibody – based inhibitors target receptor tyrosine kinases and are similar in action to receptor blockers, in the sense that signaling is blocked at the level of the receptor. Small molecule kinase inhibitors are targeted at kinases that participate in signal transduction.

The following is several kinds of protein kinases potential targets for cardiovascular disease including G – protein – coupled receptor kinases (GRKs), Ca^{2+}/calmodulin – dependent protein kinase II (CaMKII), glycogen synthasekinase (GSK) – 3β, protein kinase C (PKC), RhoGTPases, phosphoinositide 3 – kinase, mitogen – activated protein kinase and mammalian targetof rapamycin (mTOR).

3.1 G – protein – coupled receptor kinases (GRKs)

G – protein – coupled receptors (GPCRs) are widely implicated in human heart disease. The most commonly studied and clinically targeted cardiac GPCRs include the adrenergic, angiotensin, endothelin, and adenosine receptors. GPCRs mediate a number of essential events in cardiovascular function. The activation of α – and β – adrenergic, muscarinic, angiotensin II or endothelin receptors is central to cardiac contractility, vascular resistance, the development of the cardiovascular system and the growth and remodelling of different cardiovascular cell types. These receptors and their transduction systems are also the targets of many different drugs used in the treatment of angina, congestive heart failure and hypertension. All these receptors are regulated by G – protein – coupled receptor kinases (GRKs)[18]. GRKs are a family compromised of seven members, which is key participants in the pathways leading to phosphorylation – dependent GPCR desensitization, endocytosis, intracellular trafficking and resensitization as well as in the modulation of important intracellular signaling cascades by GPCR. Alterations in GRKs activity and expression have been found in diseases of cardiovascular system such as congestive heart failure or hypertension[19] and one of the GRKs family members, the GRK2 or β – ARK1 regulates β – adrenergic receptors (β – ARs) in the heart, the cardiac expression of which is elevated in human heart failure and the level of β – ARK1 inhibition can have important therapeutic implications[20]. A better knowledge of the GRK functional consequences will help us better understand the mechanisms and signals governing the expression and activity levels of different GRKs and their alterations during the progression of CVDs[19].

3.2 Ca^{2+}/calmodulin – dependent protein kinase II (CaMKII)

Recently it has become increasingly clear that several Ca^{2+} – dependent proteins contribute to the fine tuning of excitation – contraction coupling (ECC). One of these is the Ca^{2+}/calmodulin – dependent protein kinase (CaMK) of which CaMKII is the predominant cardiac isoform. CaMKII is a multifunctional, ubiquitous serine – threonine protein kinase which has been implicated in arrhythmogenesis and structural heart disease (electrical instability and arrhythmias, myocardial dysfunction, and myocardial hypertrophy and chamber dilation). CaMKII is a signaling molecule that shares many cellular targets with PKA and is activated during β adrenergic stimulation. CaMKII has been implicated in the regulation of cardiac excitation – contraction coupling (ECC) as well as in apoptotic signaling and adverse remodeling. In the I/R hearts, CaMKII inhibition reduced infarct size in association with a significant recovery of contractility during reperfusion. Moreover, CaMKII inhibition reduced LDH release, caspase – 3 activity and the number of TUNEL positive cells, and increased Bcl – 2/Bax ratio, indicating that CaMKII inhibition prevents both necrosis and apoptosis. The protective role of CaMKII inhibition against necrosis was confirmed in isolated myocytes[21]. However the present results indicate a dual role of CaMKII in the I/R and the reason for this dual effect is unknown although a similar dual action for CaMKII has

been reported in other pathological conditions[21]. Understanding CaMKII regulation in heart more completely will help to understand the normal physiology of the heart and the next few years should bring more insight into these mechanisms and possibly novel therapeutic approaches.

3.3 Glycogen synthase kinase (GSK) -3β GSK -3β is a multifunctional protein that is located at the convergence of several pro - growth signaling pathways. In myocardium, GSK -3β positively regulates apoptosis and negatively regulates hypertrophy. In blood vessels, the growth factor - PI3 - kinase - Akt axis functions downstream of GSK -3β/β - catenin signaling, in endothelial cells, to promote angiogenesis.

3.4 The protein kinase C (PKC) Several PKC inhibitors are currently available, but are not isoform specific and also target other protein kinases. Experimental data suggest that activation of some PKC isoforms may be beneficial. For example, PKC - ε appears to have a cytoprotective role in cardiac myocytes and PKC -ζ is activated in hypoxia, which acts as a terminator of ERK1/2 activation through regulation of the downstream target MKP -1, thus limiting hypoxia - induced proliferation of fibroblasts. PKC inhibitors could potentiate the vascular effects of Ca^{2+} channel blockers. Also, targeting Ca^{2+} - independent PKC isoforms could be effective in Ca^{2+} antagonist - resistant forms of hypertension.

3.5 The Rho GTPases (ROCK) ROCK is one of the downstream effectors of the small G - protein Rho. The Rho - ROCK pathway has an important role in mediating various cellular functions, including contraction, actin cytoskeleton organization, cell adhesion and motility, proliferation, cytokinesis and gene expression, all of which are involved in the pathogenesis of cardiovascular disease[22]. Abnormal activation of this pathway is associated with the pathogenesis of various cardiovascular diseases such as hypertension, coronary and cerebral vasospasm, restenosis, atherosclerosis, stroke and heart failure, although the roles of the ROCK isoforms (ROCK1 and ROCK2) remain to be elucidated[22] and ROCK inhibitors could cover the wide range of pharmacological effects of conventional cardiovascular drugs, including statins, angiotensin - converting enzyme (ACE) inhibitors, angiotensin II type 1 receptor blockers, calcium channel blockers, β - blockers and receptor blockers of thrombin, 5 - HT and endothelin, with the apparent exception of inhibitory effects on cholesterol synthesis in the liver. The available evidence indicates that these inhibitors are useful for the treatment of various disorders caused by VSMC hyperconstriction, including coronary and cerebral vasospasm, hypertension and pulmonary hypertension. They might also be useful for the treatment of various arteriosclerotic cardiovascular diseases. Several clinical studies of fasudil have indicated that ROCK inhibitors might be useful for treating cardiovascular diseases in humans, in addition to the current indication of cerebral vasospasm, including angina pectoris, hypertension, pulmonary hypertension, stroke and heart failure[22]. So far a series of pyridine derivative compounds has been developed and among them, Y27632 has shown a potent ROCK inhibitory action[23]. Y27632 selectively inhibits smooth muscle contraction by inhibiting Ca^{2+} sensitization, suppresses the ROCK - mediated formation of stress fibers in cultured cells and markedly corrects hypertension in several hypertensive rat models[23]. This pyridine derivative is used as an important pharmacological tool to examine the involvement of ROCK in the pathogenesis of cardiovascular and other diseases[22]. Recently, GSK269962A and SB772077B were characterized as members of a novel class of compounds. These compounds have similar inhibitory effects on ROCK1 and ROCK2, and their potency is higher than that of Y27632. Recently, SLx2119, which is an orally bioavailable, potent and highly selective ROCK2 inhibitor, has been developed. The 3D crystal structure of ROCK and its binding site for fasudil has been determined, which should facilitate the development of selective ROCK inhibitors[24].

3.6 The PI -3K The PI -3K family of enzymes consists of several closely related isoforms, that are classified into three subfamilies, according to structure and substrate specificity. Class I PI -3Ks have been extensively studied in the cardiovascular system. PI -3Ks are heterodimers of different catalytic domains and adaptor subunits. The class IA isoforms contain the catalytic subunits p110α, p110β and p110δ and are activated by receptor tyrosine kinases, while class IB members contain the catalytic subunit p110γ, which is acti-

vated by the β, γ subunit of heterotrimeric G proteins.

3.7 Mitogen – activated protein kinases (MAPKs), ERK1/2, JNKs, and p38MAPKs MAPK activation has been associated with rheumatoid arthritis, cancer, and cardiovascular and pulmonary diseases. A new generation of small – molecule MAPK inhibitors that demonstrate increasing specificity for each of the JNK, ERK and p38 kinase isoforms has shown promise in animal studies and could eventually prove effective for treating human diseases and several of these compounds are already being tested in human subjects to assess their oral bioavailability, pharmacokinetics and toxicity. In addition, these compounds will be useful to correlate the mechanism of action of MAPK – specific SMIs in cell culture with their SMI inhibitory – specific profiles in whole animals[25]. This could be critical for defining the future use of MAPK – specific SMIs in humans. But currently available pharmacological inhibitors do not distinguish between the p38α and β isoforms, and interfere with other MAPK pathways, particularly JNK (c – Jun N – terminal kinases). This is an important issue with respect to the use of p38 MAPK inhibitors both as a research tool and as a potential drug therapy.

3.8 Mammalian target of rapamycin (mTOR) mTOR is a serine – threonine kinase that is activated in response to growth factors, ATP and hypoxia sensors, and factors that regulate cell growth and division. Several signaling pathways that are implicated in cardiac hypertrophy, such as PI – 3K and ERK1/2, are upstream of mTOR[26].

4 Several issues in the development of protein kinase inhibitors as therapeutic agents

So far at least eight kinase – targeted oncology drugs have received regulatory approval and more than 100 additional compounds, that inhibit the activity of various protein kinases, are in clinical trials[27]. But the development of protein kinase inhibitors as therapeutic agents has confronted several issues.

Initially selectivity is a key consideration in the development of kinase inhibitors. Compounds are "profiled" for their selectivity against panels of kinases (often 30 or more) to determine which targets, aside from the intended one, are being affected. These panels are chosen in a variety of ways but often include specific kinases that one does not want the drug to inhibit and/or a selection of kinases with much structural diversity at the active site[3]. Inhibition of aberrant kinase activity in a diseased tissue could potentially inhibit beneficial activity in other tissues. For example, inhibiting the cell – surface HER2 tyrosine kinase receptor with the monoclonal antibody trastuzumab (Herceptin, Genentech) in patients with breast cancers overexpressing that receptor has produced strikingly beneficial results, but it has come at the expense of severe cardiac dysfunction in some women receiving the therapy, suggesting a critical role for this receptor in cardiomyocyte survival. This could be overcome with localized drug delivery with kinase inhibitors. Thus, inhibiting a protein kinase that is dysregulated in one organ in a particular disease state may prove harmful to other systems in which that same protein kinase is not dysregulated but instead serves essential functions. In addition to the specificity concerns there were other issues pertaining to the ubiquitousness of kinases.

Secondly the general issues are toxicity and resistant with long – term use and potency. Toxicity remains a major concern, because many of these kinases not only play roles in the pathogenesis of diseases but also function in pathways that regulate the most basic of normal cellular processes. Besides potency is critical issues for the eventual effectiveness and safety of any drug, it is expressed as the enzymatic IC_{50} (concentration of drug that inhibits enzyme activity by 50%). However, reported IC_{50s} must be interpreted with caution, because the IC_{50} determined for an ATP – competitive inhibitor will vary depending on the concentration of ATP used in the assay and on the Km (the affinity of the kinase for ATP). This has been a source of significant confusion in the literature.

5 Conclusion

Since it has proven a double – edged sword for inhibiting a protein kinase, to identify a protein kinase inhibition as a therapeutic target with fewer off – target side effects, more potency and fewer toxicity is very important. The selective inhibition of a protein kinase or protein kinases associated with a specific disease, without affecting protein kinases involved in normal physiology, remains an important goal in the design and use of protein kinase inhibitors as drugs. Off – target side effects of protein kinase inhibitors have diverted efforts

from targeting the ATP – binding pocket in order to produce inhibitors that have the potential to be more kinase specific. The requirement for selective phosphorylation of specific protein substrates by each kinase suggests that rational substrate – based design might be a promising alternative approach for the development of kinase inhibitors available for cardiovascular diseases and protein kinase inhibitors should increase the pharmacopeia. It is probably that the next several years of cardiovascular research will feature a great number of clinical trials using inhibitors of protein kinase signaling pathways to treat many CVDs.

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疾病机制与治疗:蛋白激酶及其抑制剂与心血管疾病

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[摘 要] 在当今世界上,心血管疾病是导致发病率和死亡率升高的主要原因。到目前为止,对心血管疾病的病理生理学研究与治疗的认识已经取得了实质上的进展。人体心血管系统有许多细胞信号级联放大系统,其中一些是有益的,一些是代偿的,而其它则是有害的。这些信号级联放大系统中转导途径是否平衡决定了机体有无疾病。在这其中,把细胞外刺激转导到细胞内是通过蛋白质磷酸化来完成的,而蛋白质磷酸化又被蛋白激酶介导。这种用来选择性地阻断信号转导途径的蛋白激酶抑制剂可能是一种潜在的有利的受体阻断剂。到目前为止,人们发现了各种各样的可用于治疗一些疾病的蛋白激酶抑制剂,并且用蛋白激酶抑制剂成功治疗肿瘤强有力地支持其在心血管疾病治疗中的应用。在此,我们将总结一些已经能用于心血管疾病的蛋白激酶靶位,以及一些鉴定与心血管疾病有关蛋白激酶假定的治疗靶位的难点。

[关键词] 蛋白激酶类; 蛋白激酶抑制剂; 心血管疾病

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