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Education article

G Protein Receptor Kinase Type 2: A Novel Target in Cardiovascular Drug Development

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SUMMARY

In spite of significant advancement in pharmacotherapy, heart failure remains one of the main causes of death worldwide. The existing treatment of heart failure prolongs life span by slowing down the pathological process but does not induce heart healing. The β adrenergic receptor dysfunction is a hallmark abnormality of advanced stages of heart failure, and increased expression of G-protein-coupled receptor kinase 2 (GRK-2) involved in down regulation of β adrenergic receptors, occurs in early stages of heart failure. Thus a new drug candidate, an inhibitor of G-protein-coupled receptor kinase 2 (βARKct) that prevents βAR down-regulation and desensitization, is being developed. Experimental data are encouraging.

Keywords: heart failure; β adrenergic receptor; G protein kinase 2 inhibitor

INTRODUCTION

Despite of significant progress of pharmacotherapy in last decades of the 20th century, heart failure remains a leading cause of death worldwide [1]. The quality of life and the life span are extended by the use of drugs that modify the function of the renin-angiotensin-aldosterone system, diuretics and beta adrenergic receptor blockers. Nevertheless these drugs neither induce regression nor the healing of the myocardium.

It has been largely acknowledge that the dysfunction of beta-adrenergic receptors (βAR) is the major mechanism in the development of advanced stages of heart failure. The enhanced sympathetic outflow associated with heart failure and the increased release of noradrenaline from sympathetic nerve endings in the heart, induce down-regulation and desensitization of βAR receptors. The reduction in the number of βAR in the failing heart contributes importantly to the decrease of its contractile force.

Numerous experimental findings suggest that βARKct by preventing the down-regulation of βAR improves myocardial function and reverses pathological changes. In this review, the mechanisms of βARKct action along with experimental and translational studies are described.

Methodology

Sources of studies included the Medline and SCI databases. The databases were searched from inception to October 30, 2013 for relevant studies using the terms "heart failure", "β adrenergic

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receptor", "G protein kinase 2 inhibitor" and synonyms for all three terms. Potentially associated publications were assessed by checking their titles and abstracts and the most relevant publications were subjected to closer examination. The reference lists of the selected papers were also screened for articles that might have been missed in the initial search, and references cited in the identified articles were searched manually. All eligible studies satisfied the following inclusion criteria: experimental and translational studies without language restrictions; a sample size of more than six animals/humans was required for each comparison group. Studies were excluded based on the following criteria: studies not mentioning G protein kinase 2 inhibitor in the abstract; studies without comparison groups; abstracts presented at conferences, editorials, commentaries, and studies without complete data; cohort studies and case-control studies were excluded.

BETA-ADRENERGIC RECEPTORS AND THE SIGNALING PATHWAY IN THE HEALTHY HEART

βAR is a classical transmembrane receptor with seven transmembrane domains, an amino terminus on the extracellular surface and carboxyl tail on the intracellular surface of the cardiomyocyte. Binding of natural ligands to βAR such as adrenaline, noradrenaline, dopamine, or beta-adrenergic agonists such as dobutamine leads to G protein activation. G protein is an intracellular heterotrimer with α, β, γ subunits. α subunit activates adenylyl cyclase that promotes synthesis of cyclic adenosine monophosphate (cAMP) and the activation of protein kinase A (PKA). PKA phosphorylates phospholamban (cell protein) to prevent calcium-ATPase inhibition that will enable calcium extrusion from sarcoplasmic reticulum and recruit more muscular fibers in contraction, a mechanism underlying positive inotropism.

Under physiological conditions, binding of agonist to βAR triggers intracellular mechanisms leading to βAR desensitization (Figure 1). Desensitization involves G protein receptor kinase 2 binding to βγ subunit (Gβγ) of G protein and its translocation in the membrane of cardiomyocytes, where it phosphorylates βAR. Phosphorylation changes the βAR conformation that allows the bind of β arrestin, clathrin and adaptin. These cytoplasmic proteins act to invaginate the cell membrane and enclose βAR in the vesicle while dynamin segregates the vesicle from the membrane to form endosomes, a process well known as endocytosis (Figure 2). The faith of the internalized receptors in endosomes depends on many factors and two pathways are possible: quick recycling, i.e. re-incorporation in the membrane or degradation by fusion with lysosomes.

BETA-ADRENERGIC RECEPTORS (βAR) AND THE SIGNALING PATHWAY IN THE FAILING HEART

In the myocardium, there are three types of βAR: βAR1, βAR2 and βAR3 in the ratio 80:18:2. While βAR1 receptors mediate effects of catecholamine on the heart, βAR2 receptors

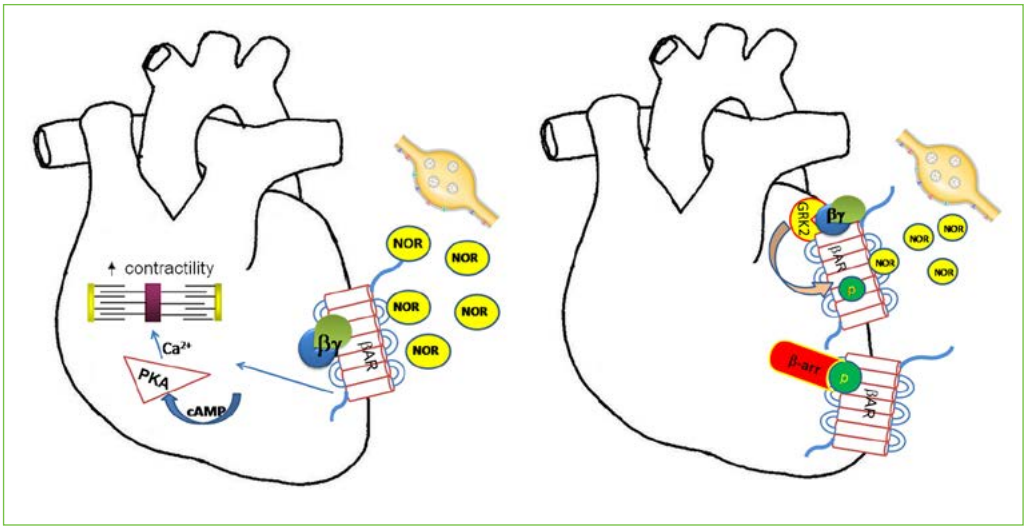

Figure 1. Signaling of βAR

Left: After agonist binding to βAR, Gα subunit stimulates the synthesis of cyclic adenosine monophosphate (cAMP) leading to enhanced myocardial contractility.

Right: GRK-2 kinase binds to Gβγ subunit to translocate GRK-2 in the membrane and phosphorylate βAR leading to binding of β-arestin (β-arr).

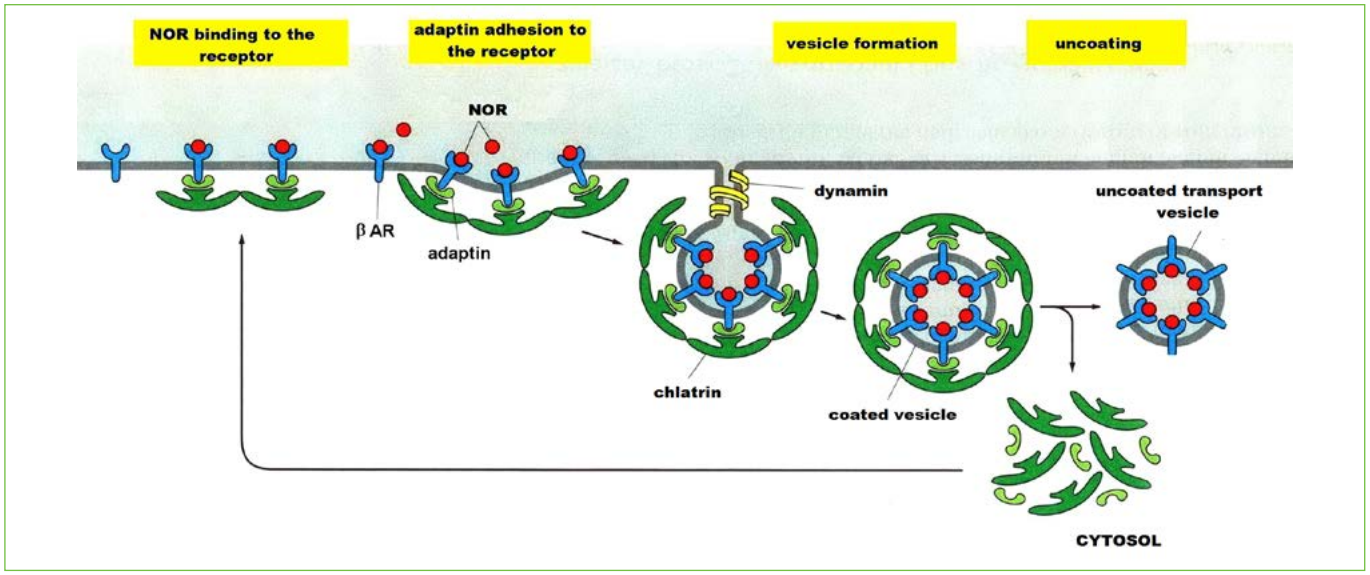

Figure 2. βAR internalization

Agonist binding to βAR activates adaptin and its binding to the intracellular domain of βAR. Then clatrin binds to adaptin to form a vesicle and internalize βAR (modified from [32]).

are linked to inhibitory G protein and are considered to exert protective effects on cardiomyocytes [2, 3, 4]. It has been suggested that in the failing heart, the change in βAR1:βAR2 to 50:50 ratio, and the change in the conformation of βAR receptors, contribute to apoptotic mechanism. Even in early stages of heart failure, due to the development of hyperadrenergic state [5-9] and increased stimulation of βAR by catecholamines [10-13] boost up GRK-2 expression leading to βAR1 receptor desensitization [5, 6, 14, 15]. In the time course of the disease, the reduced number of βAR1 will decrease its contractile force [11]. Therefore the increase of GRK-2 expression has been proposed as an early marker of heart failure [16, 17, 18].

GRK-2 inhibitor (βARKct) in experimental models of heart failure

GRK-2 inhibitor is the carboxyl part of the GRK-2 chain. Its mechanism of action is based on the competitive binding to βγ subunit of G protein and displacement of GRK-2 from the Gβγ.

The role of GRK-2 in the development of heart failure was demonstrated in genetic experimental models of heart failure. In knockout (KO) mice deletion of the gene for the muscle LIM domain protein – MLP [19] induce heart failure. Also, over-expression of *calsequestrin, a* calcium binding protein in mice induces heart failure [20]. Administration of βARKct to both models of KO mice prevented the development of heart failure and extended the life span of KO mice. Moreover, experimental data indicate that co-administration of beta-blockers and βARKct had synergetic beneficial effects in experimentally-induced heart failure [20].

Deletion of GRK-2 gene in mice has been shown to be associated with embryonic death [21], indicating that GRK-2 is essential for heart organogenesis. In heterozygous GRK-2 KO mice (which have 50% expression of GRK-2 genes) concomitantly transfected with βARKct, the contractile force of the heart and βAR reserve were enhanced [22]. In heterozygous GRK-2 KO mice exposed to anterior coronary artery ligation (an experimental model of myocardial necrosis) a mortality rate was reduced [23]. In the same study, GRK-2 gene deletion was demonstrated to be more effective than pharmacological blockade of βAR.

TRANSLATIONAL STUDIES – FROM ANIMALS TO HUMANS

βARKct has a negative ADME (adsorption, distribution, metabolism and excretion) profile and is thus hardly deliverable to the heart cells both *in vivo* and *in vitro* (Figure 3). Therefore, vectors modified non replicant viruses, whose genome contain a therapeutic gene instead of the protein capsid gene, were used to deliver

βARKct to cells. Adenoviral transfection with βARKct in cardiomyocytes has been shown to be effective in animal models of heart failure [24, 25, 26]. Adenoviral βARKct gene delivery by intra-cardiac injection to rabbit hearts in experimentally-induced necrosis of myocardial tissue by ligation has been shown to prevent remodeling of left ventricle [27, 28]. Two protocols of gene therapy in humans, in 2008 [29] and in 2009 [30] indicate that adenoviral and adeno-associated viral transfections are not harmful to humans.

New indication for an old drug?

Paroxetine is an antidepressant drug, selective serotonin inhibitor, registered worldwide since 1992. Recently, a group of researchers from Michigan, New York and Pennsylvania [31] identified it as a potent direct inhibitor of GRK-2. Maximal GRK-2 inhibition by paroxetine was reported to be at doses higher by 1-2 powers on log scale. Experimental data showed that paroxetine increases cardiac inotorpism both *in vitro* and *in vivo* while fluoxetine, a related drug, does not because it is deprived of the ability to inhibit GRK-2. In the post-marketing period of paroxetine, there were reports that mothers treated with paroxetine during pregnancy, delivered children with cardiovascular defects, suggesting that this teratogenic effect of paroxetine could be related to the inhibition of GRK-2. Thus, clinical studies aimed at evaluating the outcome of patients suffering from heart failure on chronic paroxetine treatment (phase IV clinical trials) are desirable.

CONCLUSIONS

G protein kinase-2 inhibitor is a promising novel target for new drug development in advanced stages of heart failure associated with down regulation of β adrenergic receptors. The use of the peptide inhibitor is hindered by the development of more effective and harmless gene delivery systems. Meanwhile, the investigation of the effectiveness of paroxetine in heart failure (phase IV clinical studies) and the synthesis of related molecules may be a quicker way to approach the development of new drugs.

Conflict of Interest Statement

Authors declare no conflict of interest.

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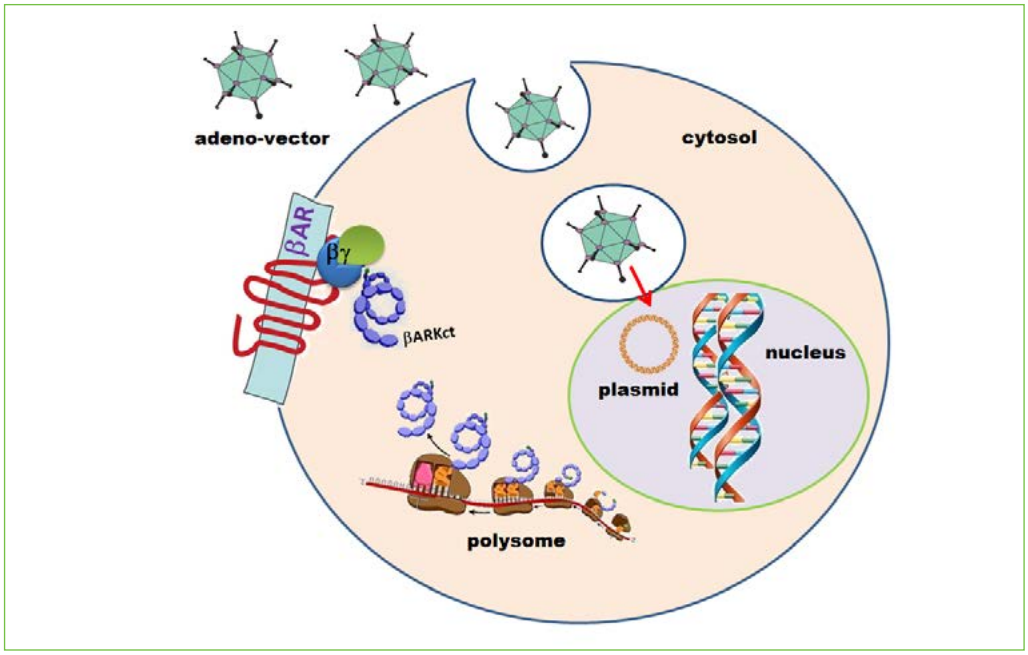

Figure 3. Adenoviral transfection

Adenoviral vector infects cardiomyocyte by endocytosis. Its plasmid reaches the nucleus and then starts the transcription of β ARKct.

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Inhibitori G-protein kinaze 2 u lečenju slabosti srca

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KRATAK SADRŽAJ

Pored značajnog napretka farmakoterapije, insuficijencija srca ostaje jedan od vodećih uzroka smrti ljudi. Postojeća farmakoterapija produžava život čoveka, ali ne dovodi do izlečenja. Ključnu ulogu u nastanku uznapredovale insuficijencije srca ima disfunkcija beta adrenergičkih receptora (βAR) koja se podudara s povećanjem ekspresije G-protein kinaze tip 2 (GRK-2). Novi kandidat za lek u terapiji osoba s insuficijencijom srca je inhibitor GRK-2 (βARKct), koji sprečava nishodnu regulaciju βAR u insuficijenciji srca. U radu su prikazani najnoviji eksperimentalni nalazi koji obećavaju.

Ključne reči: insuficijencija srca; beta adrenergički receptor; inhibitor G-protein kinaze 2

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