Commentary
β-Adrenergic gene therapy for cardiovascular disease
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Abstract

Gene therapy using *in vivo* recombinant adenovirus-mediated gene transfer is an effective technique that offers great potential to improve existing drug treatments for the complex cardiovascular diseases of heart failure and vascular smooth muscle intimal hyperplasia. Cardiac-specific adenovirus-mediated transfer of the carboxyl-terminus of the β-adrenergic receptor kinase (βARKct), acting as a $G_{\beta\gamma}$–β-adrenergic receptor kinase (βARK)1 inhibitor, improves basal and agonist-induced cardiac performance in both normal and failing rabbit hearts. In addition, βARKct adenovirus infection of vascular smooth muscle is capable of significantly diminishing neointimal proliferation after angioplasty. Therefore, further investigation is warranted to determine whether inhibition of βARK1 activity and sequestration of $G_{\beta\gamma}$ via an adenovirus that encodes the βARKct transgene might be a useful clinical tool for the treatment of cardiovascular pathologies.

Keywords: adrenergic receptors, gene therapy, heart failure, intimal hyperplasia

Introduction

Heart failure and hypertension are primary sources of morbidity and mortality in developed countries. There are numerous pharmaceutical agents on the market today that are used to treat these complex diseases. However, such agents are still not able to cure or to ameliorate symptoms for all patients. For this reason, new drugs and therapies are constantly being sought [1].

Gene therapy that uses *in vivo* recombinant adenovirus-mediated gene transfer is a powerful technique that offers great potential to improve existing drug treatments. It allows for transient expression of gene products such as peptides in targeted tissue, and therefore has the potential to be a highly specific treatment. Adenovirus-mediated gene therapy has recently come under assault because of the potentially harmful inflammatory responses to the high doses of adenovirus vectors [2]. It is likely that existing vectors will need to be improved for successful application in humans, although this is not a focus of the present commentary and readers are referred to more recent detailed discussions of viral vectors [2–5].

Gene therapy and cardiovascular disease

Several recent animal studies provide compelling evidence that gene therapy will be of great benefit as a

βAR = β-adrenergic receptor; βARK = β-adrenergic receptor kinase; βARKct = carboxyl-terminus of the β-adrenergic receptor kinase.

treatment for cardiovascular diseases. For example, gene therapy using transgenes that enhance the contractile function of the heart have the potential to act as a therapeutic bridge before and during cardiac transplantation. Moreover, gene therapy may be able to ameliorate complications that arise throughout critical periods of cardiovascular disease processes, such as the remodeling that occurs after myocardial infarction or the restenosis that can occur after angioplasty or stent implantation to correct blocked arteries. Therapeutic agents aside, cardiovascular gene transfer provides an invaluable tool for examining disease-based mechanisms in animal models. Results of studies from our laboratory and from others suggest that gene therapy could become a very powerful therapeutic strategy in the very near future.

Delivery of transgene

One hurdle that must be overcome in order for gene therapy to become successful in humans is a clinically relevant, reliable, and specific method of delivery of the transgene to the cardiovascular system. Several groups have demonstrated the feasibility of delivering transgenes to the heart via direct intramyocardial injection [6,7], *ex vivo* perfusion [8–10], and finally an intracoronary approach [11–14]. Direct injection of the adenovirus into the ventricular wall has been shown to induce significant expression of transgenes with a limited amount of adenovirus [6,7]. However, there is needle damage and expression is limited to the site of injection.

Although limited in scope, *ex vivo* delivery systems, such as during cardiac transplantation, are clinically relevant and allow for global expression of different transgenes [8–10]. In order to accomplish expression of the transgene, the aortic root of the donor heart is perfused with adenoviral solution and then heterotopically transplanted into the recipient [9]. This method has been used to target robust myocardial overexpression of β_2-adrenergic receptors (β_2ARs), which translated into a functional effect on the transplanted heart in the form of significantly enhanced basal and agonist-induced contractility [10]. Expression of the β_2AR transgene was transient, and peak expression was seen at 5 days and returned to baseline levels after 14 days [9]. This method may provide a unique opportunity for genetic manipulation of the donor organ, potentially enhancing its function during the stresses of open-heart surgery. In addition, because gene delivery is carried out in the donor heart, total body adenovirus exposure is limited in the recipient, thus making it clinically attractive for the vectors that are now available.

Another successful method of gene transfer to the heart is injection of adenovirus through the coronary arteries. Recently, investigators from two laboratories, including ours, have developed a method for global myocardial transgene delivery [12,13]. This method involves injecting adenovirus into the left ventricular cavity while the aorta or aorta and pulmonary artery are cross-clamped for several seconds, allowing perfusion of the coronary arteries via the aortic root. We have shown that *in vivo* myocardial gene delivery in rabbits using this left ventricular/cross-clamp delivery method of either the β_2AR transgene or a gene that encodes a peptide inhibitor of the βARKct can enhance cardiac function in normal hearts as well as failing hearts [13,15]. The βARKct is comprised of the last 194 amino acids of bovine βARK1, which contains the region for binding to $G_{\beta\gamma}$ ($G_{\beta\gamma}$-subunits of activated heterotrimeric G proteins), a process that is required for βARK1 activation [15]. Thus, the βARKct inhibits the activity of βARK1 through the competitive binding of $G_{\beta\gamma}$. Global *in vivo* myocardial gene delivery via this left ventricular/aortic cross-clamp technique has been quite successful. It is quite invasive, however, because it requires thoracotomy; thus, less invasive methods are desirable.

Recently, it has become possible to deliver therapeutic adenoviral transgenes to the beating rabbit heart *in vivo* via a percutaneous intracoronary catheterization injection [14]. This technique supports transgene delivery to the area of the heart that is supplied by the catheterized artery, and may be advantageous when global transgene delivery is not required. This percutaneous coronary catheterization technique does not require entrance in the thoracic cavity, and is therefore an attractive and clinically relevant technique for use in heart failure treatment. We delivered the β_2AR transgene in a ventricular-specific manner after right coronary artery or left circumflex coronary artery catheterization and adenovirus injection, and found that *in vivo* ventricular contractility can be enhanced 5 days after this gene treatment [14].

β-Adrenergic receptor signaling and heart failure

The use of βAR signaling components as gene therapy for heart failure has been the subject of debate for the past couple of years [16]. It is clear that alterations in the myocardial βAR system both precede and accompany the development of heart failure in humans [17,18]. These alterations include a 50% decrease in β_1ARs, and functional uncoupling of remaining β_1ARs and β_2ARs in the myocardium. The latter is probably mediated by a significant upregulation in the expression and activity of βARK1 [19]. Importantly, previous work in our laboratory using novel genetically engineered mice has clearly shown that enhancing βAR signaling through overexpression of β_2ARs or inhibiting βARK1 via the βARKct are novel therapeutic approaches to enhance the function of the heart [20]. In fact, this strategy has prevented the development of cardiomyopathy in murine models of heart failure [20]. Thus, data in transgenic mice coupled with our initial gene therapy results in rabbits demonstrate that enhancing myocardial β-adrenergic signaling either by overexpressing

β_2ARs or by inhibiting βARK1 activity may provide novel molecular ventricular assistance for failing hearts.

β-Adrenergic receptor signaling and vascular intimal proliferation

Not only has gene therapy with βAR signaling components been successful for treating animal models of heart failure, but we have also shown that it can be used successfully for vascular pathologies as well [21,22]. Functional expression of transgenes including the β_2AR in vascular smooth muscle has been demonstrated using catheter-mediated adenovirus delivery to de-endothelialized rat carotid arteries [22]. Total βAR density was increased in the carotids after β_2AR adenovirus treatment, and this resulted in an enhancement in isoproterenol-induced vasorelaxation [22]. In humans, this could be used to improve blood flow specifically to ischemic regions, and may also lead to novel therapeutic strategies for treatment of hypertension.

Percutaneous transluminal coronary angioplasty is a method that is employed to improve cardiac blood flow through compromised vessels in a number of diseases, including unstable angina and myocardial infarction [23]. However, at the site of angioplasty there is significant injury to the arterial wall, and a major limitation to this technique is induction of the accumulation and proliferation of vascular smooth muscle cells from the tunica media to the tunica intima of the arterial wall. This proliferation leads to restenosis in 30–60% of cases within 3–6 months and is a clinical process known as intimal hyperplasia [24]. Because adenovirus-mediated transfer of a functional gene to vascular smooth muscle is feasible, we have been interested in determining whether we could prevent the smooth muscle intimal hyperplasia by directing transgene expression at the site of injury. Interestingly, it appears that, for several mitogenic agents that signal through G-protein-coupled receptors, $G_{\beta\gamma}$ is a critical signaling component for mitogenesis and cell proliferation. However, this has never been documented for *in vivo* vascular smooth muscle intimal hyperplasia. Thus, we have utilized the βARKct transgene as an inhibitor of $G_{\beta\gamma}$ in a rat carotid balloon-catheter angioplasty injury model in order to investigate the role of $G_{\beta\gamma}$ in this pathologic event [21]. As detailed above, because the βARKct inhibits βARK activity through competitive $G_{\beta\gamma}$ binding, it serves as a specific $G_{\beta\gamma}$ inhibitor. Importantly, we found that $G_{\beta\gamma}$ plays a major role in vascular smooth muscle proliferation, because targeted inhibition of $G_{\beta\gamma}$ by the βARKct resulted in a significant reduction in intimal hyperplasia in this rat model of arterial restenosis [21].

Conclusion

Experimental data strongly suggest a key role for the βAR signaling system in the pathogenesis of cardiovascular disease. In heart failure, increasing βAR signaling through genetic inhibition of βARK1 or overexpression of β_2AR improves cardiac performance by providing molecular ventricular assistance, and may represent a novel therapeutic approach to the management of heart failure. In addition, smooth muscle intimal hyperplasia is prevented with the expression of βARKct. Therefore, vascular pathologies are also dependent on G-protein-coupled receptor signaling. Work from our laboratory and from others suggests that adenovirus-mediated gene therapy is a powerful technique for the selective, tissue-specific expression of gene products in the cardiovascular system. To date, pharmaceutical agents are able to achieve this, and current management of both heart failure and intimal hyperplasia is not capable of preventing or completely alleviating symptoms and mortality. Therefore, we remain optimistic that adenovirus-mediated gene therapy and, more specifically, the inhibition of βARK1 activity and sequestration of $G_{\beta\gamma}$ via the βARKct will be an invaluable clinical tool for the treatment of cardiovascular pathologies.

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