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Protein Therapeutics for Cardiac Regeneration after Myocardial Infarction

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Abstract

Although most medicines have historically been small molecules, many newly approved drugs are derived from proteins. Protein therapies have been developed for treatment of diseases in almost every organ system, including the heart. Great excitement has now arisen in the field of regenerative medicine, particularly for cardiac regeneration after myocardial infarction. Every year, millions of people suffer from acute myocardial infarction, but the adult mammalian myocardium has limited regeneration potential. Regeneration of the heart after myocardium infarction is therefore an exciting target for protein therapeutics.

In this review, we discuss different classes of proteins that have therapeutic potential to regenerate the heart after myocardial infarction. Protein candidates have been described that induce angiogenesis, including fibroblast growth factors and vascular endothelial growth factors, although thus far clinical development has been disappointing. Chemotactic factors that attract stem cells, e.g. hepatocyte growth factor and stromal cell derived factor-1, may also be useful. Finally, neuregulins and periostin are proteins that induce cell cycle reentry of cardiomyocytes, and growth factors like IGF-1 can induce growth and differentiation of stem cells. As our knowledge of the biology of regenerative processes and the role of specific proteins in these processes increases, the use of proteins as regenerative drugs could develop as a cardiac therapy.

Keywords

protein therapeutics; myocardial infarction; regeneration; heart failure

Cardiovascular diseases are a leading cause of death worldwide, and it has been projected that cardiovascular deaths will increase from 16.7 million in 2002 to 23.3 million in 2030 due to aging populations [1]. Ischemic heart disease including acute myocardial infarction (MI) will remain an important cause of heart failure and mortality despite our successes in this disease. MI typically results in death of millions of myocytes which are replaced by non-contractile scar tissue in the weeks following the infarct. This results in an increased mechanical load on surviving myocytes, inducing a remodeling process which over time can progress to clinical heart failure.

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Conflicts of interest

Vincent Segers and Richard Lee are founders of Provasculon Inc.

The current standard of care for MI is early reperfusion of the occluded vessel with angioplasty or thrombolysis to reverse ischemia and increase the number of surviving myocytes. Efforts to decrease delays between onset of symptoms and reperfusion have resulted in decreased morbidity and mortality, but the maximal benefit of early reperfusion has reached a point close to practical limits [2]. Besides early reperfusion therapy, ACE inhibitors and beta-blockers are used to prevent remodeling after MI and progression to heart failure. Both ACE inhibitors and beta-blockers improve long term survival but no therapies besides cardiac transplantation are currently available that restore cardiac function.

In the last decade, a large number of pre-clinical and clinical studies have been published on the potential use of stem cells for cardiac regeneration after MI [3]. Different stem cell types have been shown to improve cardiac function in animal studies and can induce a small but potentially significant increase in ejection fraction in clinical studies [3]. Stem cell therapy is a promising treatment option for heart failure, but numerous technical challenges and gaps in our understanding of stem cell behavior may limit translation to the clinic [3].

With the advent of biotechnology, protein and peptide drugs are becoming increasingly important in modern medicine. Drugs based on naturally-occurring proteins have the advantage of efficacy based on a mechanism of action refined by millions of years of biological evolution. Though promising as therapeutics, proteins might behave differently when used at pharmacological instead of physiological concentrations with an increase in adverse effects on other organs. Proteins used as therapeutics have been modified in different ways to limit immunogenicity and rapid degradation in plasma and tissues [4,5].

In this review, we discuss four different classes of proteins that could potentially benefit patients with MI (Figure 1); all of these proteins have been shown to improve cardiac function in animal models of MI or heart failure. They include angiogenic growth factors, proteins that increase recruitment of progenitor cells to the heart, proteins that induce mitosis of existing myocytes, and proteins that increase differentiation and growth of stem cells and myocytes. As more is learned about cardiac regeneration and why mammals lack sufficient myocardial regeneration, more proteins are likely to be added to this list of candidates.

Angiogenic growth factors

Angiogenic growth factors for treatment of MI and chronic myocardial ischemia have been studied in animals for decades [6,7]. Occlusion of the coronary arteries not only leads to defects in large vessels but also damages endothelial cells of the microvasculature [8]. Treatment of myocardial ischemia with angiogenic growth factors can increase perfusion of the heart and preserve cardiac function (Figure 1A). Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) are potent growth factors for endothelial cells [9] and can induce angiogenesis and improve cardiac function when administered after MI [7,10].

Although angiogenic growth factors have been studied extensively and many preclinical studies have reported efficacy of VEGF or FGFs for inducing angiogenesis after MI, neither VEGF nor FGFs have been successfully used as protein drugs for treatment of MI in clinical practice. One reason for failed translation to patients was that both growth factors induce nitric oxide-mediated hypotension when injected in coronary arteries, both in pigs [11] and in humans [11,12] limiting the maximal dose that can be delivered to 50ng/kg/min for VEGF [12]. In a randomized trial, intracoronary delivery of this dose of VEGF in patients with stable angina failed to meet the primary endpoints [13]. Another drawback of angiogenic growth factors is the formation of aberrant and leaky vessels [9,11,14]. This phenomenon may have been more pronounced with gene therapy approaches used to deliver VEGF in initial studies [15]. Gene therapy allows for long-term delivery but with limited control of local pharmacokinetics. Formation of stable, non-leaky vessels might require the concerted action of different growth

factors and factors that are traditionally considered anti-angiogenic (e.g. angiopoietins) [16]. Administration of different proteins, in different concentrations at different points in time is challenging for drug development programs but might be necessary for successful recapitulation of angiogenic biology.

Another reason for failed translation to patients could be the complexity of the VEGF signaling system: many different isoforms of VEGF exist that bind to a number of different receptors [9,17,18]. To date, it is unclear whether certain isoforms have better pharmacokinetic/ pharmacodynamic profiles than other isoforms for the treatment of MI. Furthermore, FGFs are growth factors that stimulate mitosis of many different cell types which can trigger tumor growth [11]. VEGF stimulates mitosis of endothelial cells more specifically, but can promote tumor expansion by stimulating angiogenesis in larger tumors [9,11].

To limit the side effects of angiogenic growth factors outside the heart, targeted delivery of growth factors specifically to the ischemic myocardium could be useful. For instance, Scott et al. targeted VEGF to the ischemic myocardium using anti-P-selectin coated liposomes as a delivery vehicle [19]. Because P-selectin is upregulated on endothelial cells in ischemic myocardium, VEGF was concentrated in the ischemic heart after systemic injection and increased cardiac function after MI [19]. Targeting proteins to the ischemic myocardium allows for straightforward systemic injection of the proteins while reducing off-target side effects.

Proteins that increase recruitment of progenitor cells to damaged myocardium

In the last decade, different stem and progenitor cells have been identified that can regenerate infarcted myocardium, both outside – most notably the bone marrow – and inside the heart [3]. Despite the fact that the true regenerative potential of these putative stem cells is still controversial, many preclinical and clinical studies have been performed with progenitor cells delivered locally, either by injection in the coronary artery or direct injection in the myocardium [3]. Injection of bone marrow stem cells has been shown to induce a modest but reproducible increase in cardiac function in clinical trials [3]. Progenitor cells can be isolated from the heart itself [20–22], and it has been shown that a limited number of new myocytes are formed in a mouse model of MI [23]. This suggests that the heart contains cells that can form new myocytes after MI, but are insufficient in number or are insufficiently activated to induce significant regeneration. An alternative approach to harvesting and transplanting progenitor cells in the heart is the use of proteins that attract progenitor cells to the site of injury (Figure 1B).

Granulocyte colony stimulating factor (G-CSF) is a growth factor that stimulates proliferation of hematopoietic stem cells and is widely used in clinical hematology. Cardiologists became interested in G-CSF when it was suggested that stem cells mobilized with G-CSF could regenerate infarcted myocardium in mice [24] (Table 1). Because G-CSF was already approved by the Food and Drug Administration for use in patients, the first clinical trials started shortly after, with mixed results [25,26]. A recent meta-analysis of clinical studies using G-CSF after MI suggested no beneficial effect on ejection fraction in the overall study population [27], but subgroup analysis revealed an increase of 4.75% in ejection fraction when G-CSF therapy was started within 37 hours after MI [27]. No differences in mortality or vessel restenosis were observed [27]. Only 385 patients were included, but this meta-analysis indicates that therapy with G-CSF is safe and has a modest effect on cardiac function when administered early after MI [27]. Until now, G-CSF was the only protein used in clinical studies that could play a role in regeneration of the heart after MI. However, the myogenic differentiation potential of the cell type mobilized by G-CSF – e.g. hematopoietic stem cells – is controversial and generally assumed to be low [3]. It has also been shown that G-CSF has anti-apoptotic properties on cardiomyocytes after MI [28], which supports the finding that

treatment with G-CSF early after MI improves ejection fraction whereas late treatment does not [27].

Hepatocyte growth factor (HGF) was first identified as a mitogenic growth factor for hepatocytes [29], but it also has chemotactic and antiapoptotic activities on multiple cell types [30]. Intravenous administration of hepatocyte growth factor after ischemia/reperfusion injury in rats decreases cardiomyocyte apoptosis and infarct size [31]. Besides this antiapoptotic effect of HGF on cardiomyocytes, HGF might also influence cardiac function after MI by its chemotactic effects on cardiac progenitor cells. The tyrosine kinase receptor of HGF – c-Met – is expressed on different populations of putative cardiac stem cells including c-Kit positive cells [20], and Sca-1 positive stem cells [21]. HGF has been shown to be chemotactic for c-Kit+ cardiac stem cells [32] and to increase formation of new myocytes when injected in infarcted myocardium together with IGF-1 [32]. Administration of HGF has been reported to improve cardiac function after MI and decrease infarct size by a number of different mechanisms including progenitor cell recruitment [32], angiogenesis [33], and anti-apoptotic effects [31] (Table 1). It remains unclear which of these mechanisms is the most important for the cardioprotective effect of HGF. For successful translation to clinical application of a protein, a clearly defined primary mode of action and knowledge on pharmacokinetic properties are necessary for the rational development of the protein as a therapeutic (Table 2).

Stromal cell derived factor-1 (SDF-1) is a small protein of 68 amino acids that is a member of the chemokine family. SDF-1 was first identified as the crucial homing signal for hematopoietic stem cells to the bone marrow after stem cell transplantation [34]. SDF-1 is highly conserved across species, and deletion of SDF-1 or its receptor CXCR4 is lethal in mice [35,36] indicating the importance of SDF-1 in mammalian stem cell biology and cardiac development. SDF-1 is a candidate for cardiac therapy because it attracts endothelial progenitor cells [37]. SDF-1 expression is upregulated after MI [38], but expression is either too low or too short to induce a significant increase in regeneration of the vasculature. Early studies using gene therapy for prolonged delivery to the myocardium [39] showed that a sustained local concentration of SDF-1 after an acute MI increases vessel density and improves cardiac function. SDF-1 is rapidly inactivated by matrix metalloproteinases [40] including MMP-2, which is highly upregulated after MI during the cardiac remodeling phase and by DPPIV/CD26. Therefore, one time administration of SDF-1 protein might be insufficient for angiogenesis after MI. A variant of SDF-1, called SSDF-1(S4V), was designed with a valine for serine substitution at position 4, which protects against cleavage by MMP-2, and with an extra serine at the N-terminus which protect against DPPIV [41]. Delivery of this protease resistant SSDF-1 (S4V) with self-assembling peptide nanofibers increased cardiac function and vessel density in a rat MI model [41]. Self-assembling nanofibers in this study allowed for sustained local concentrations, while protease resistance allowed for prolonged activity of SDF-1.

Although SDF-1 increases angiogenesis in multiple disease models and in different tissues, SDF-1 may be acting not as a growth factor on endothelial cells but as a chemoattractant for endothelial progenitor cells [37]. This unique feature of SDF-1 not only makes it a powerful inducer of angiogenesis, it also might limit the potential adverse effects when used as a therapy. As discussed in the previous section, VEGF also induces angiogenesis, but multiple studies have shown that a high local concentration of VEGF can induce uncontrolled proliferation of endothelial cells with formation of tortuous and structurally abnormal vessels [9,14].

A decade of extensive research on cardiac stem cell biology revealed 1 protein (G-CSF) that can be used to mobilize hematopoietic stem cells and just 2 proteins with chemotactic properties on stem cells: SDF-1 on endothelial progenitor cells and HGF on cardiac stem cells. Another protein that has been identified as a stem cell attractant is monocyte chemotactic protein-3 which attracts mesenchymal stem cells [42]. It is unknown if local administration of MCP-3

improves cardiac function. Identification of new stem cell chemotactic proteins is important because it could lead to the development of new and feasible therapeutics for treatment of MI and heart failure. At the same time, the true regenerative potential of most stem cells remains highly controversial; indicating that even if a chemotactic factor attracting stem cells to the heart is identified, formation of functional myocardial is still a challenging task.

Proteins that induce proliferation of myocytes

Mammalian cardiomyocytes have long been considered terminally differentiated cells which, similar to neurons, cannot undergo mitosis. It is now clear that turnover of myocytes occurs in adult hearts [43,44] but at a much lower rate than observed in neonatal hearts or in adult hearts of certain vertebrates like newt or zebrafish [45,46]. Recent reports have suggested that some proteins, including periostin [47] and neuregulin [48], can induce cell-cycle reentry in adult cardiomyocytes (Figure 1C).

Periostin is a 90 kDa extracellular matrix protein expressed by fibroblasts that plays an important role during cardiac development and in epithelial-mesenchymal transition [49–51]. During cardiac development, periostin guides cellular trafficking and extracellular matrix organization [51]. Periostin expression is minimal in adult hearts but is upregulated after injuries including MI [51] where it plays a role in remodeling of the extracellular matrix. Mice deficient in periostin show defective scar formation leading to increased frequency of ventricular rupture in the first days after MI [52]. Kuhn et al. suggested that periostin induces reentry of adult myocytes into the cell cycle in vitro and in vivo [47]. Periostin was delivered to infarcted myocardium in rats with a patch of Gelfoam [47], which is an extracellular matrix preparation currently used as a surgical device promoting hemostasis. Periostin delivered in this manner improved cardiac function after MI and decreased scar formation and remodeling of non-infarcted myocardium [47]. Periostin induced reentry into the cell cycle of 0.6 to 1% of myocytes in the border zone of the MI [47]. The results of Kuhn et al. are interesting because they could lead to a novel approach for the treatment of MI by stimulating mitosis of surviving myocytes in the border zone of the infarct. However, this hypothesis is still controversial, and Lorts et al. did not show an increase in mitosis of myocytes by periostin [53]. Potential reasons for the differences between these 2 papers [47,53] are the use of a truncated form of periostin compared to gene overexpression, the use of rats compared to mice, and acute vs. chronic administration by respectively Kuhn et al. [47] and Lorts et al. [53].

Another protein that recently has gained interest as a possible mitogen for myocytes is **neuregulin**. This member of the epidermal growth factor family stimulates survival, proliferation and differentiation of many different cell types, including myocytes [54]. Neuregulin is expressed in endocardial endothelium of neonatal hearts and is an important mediator of trabeculation and cushion formation in cardiac development [54]. In the adult heart, neuregulin is mainly expressed in endothelial cells and stimulates cardiomyocyte survival and hypertrophy [55]. Many different splice variants of neuregulin exist, with both secreted and membrane bound variants [54]. Bersell et al. have shown that neuregulin can induce cell-cycle reentry of adult mouse myocytes [48]. Daily intraperitoneal administration of ~ 0.1mg/kg neuregulin increased cardiac function after MI with a decrease in infarct size [48]. The authors showed that this was due to proliferation of existing myocytes rather than a decrease in myocyte apoptosis or an increase in progenitor cell differentiation. On condition that the mitotic effects can be translated to human adult cardiomyocytes, neuregulin could be a promising treatment because it can be administered systemically.

Theoretically, proteins like periostin and neuregulin which stimulate mitosis of surviving myocytes can partially restore the damage inflicted by MI. However, some requirements have to be met before this will result in a viable therapy. The molecule has to be specific for myocytes

to prevent tumor formation and growth in other tissues. An inherent selectivity for myocytes would also allow for systemic delivery as opposed to the use of more complicated local delivery methods. An important factor to consider is the duration of the signal necessary to induce mitosis in a significant number of myocytes. A protein that induces cell cycle reentry in a significant fraction of myocytes with a single pulse has more therapeutical potential than a protein that needs sustained or repeated delivery. Ideally, pro-mitotic proteins will be not only specific for myocytes in general but might also be specific for myocytes in the border zone of the MI. It is unlikely that uncontrolled proliferation of myocytes in the remote zone leading to myocardial hyperplasia will benefit the function of myocardium following MI, considering that cardiomyocyte hypertrophy resulting in excessive myocardial tissue hypertrophy leads to adverse remodeling. Also, formation of new myocytes, either by stem cell differentiation or by myocyte mitosis, carries an increased risk of ventricular arrhythmias.

Proteins that induce growth and differentiation of stem cells

Besides the attraction of multipotent progenitor cells to the damaged myocardium, an important goal of therapeutic cardiac regeneration is survival, growth and differentiation of these stem cells [3] (Figure 1D). **Insulin like growth factor-1** (IGF-1) is a key protein in skeletal and cardiac muscle development of which many isoforms exist that have distinct biological functions [56,57]. IGF-1 induces proliferation and survival of cardiac stem and progenitor cells and improves cardiac function [58,59]. Furthermore, addition of IGF-1 to transplanted cells enhances maturation of stem cells to larger myocytes compared to transplantation of stem cells alone [58].

Induction of differentiation of stem cells – including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPS) cells – into cardiomyocytes in vitro is accomplished by embryonic body formation or co-culture with stroma cells [60–62]. In contrast to the induction of differentiation of embryonic stem cells into endothelial cells by VEGF [62], induction of differentiation of ESCs into cardiomyocytes requires a well orchestrated spatial and temporal distribution of many different proteins [63]. Members of bone morphogenic proteins (BMPs), transforming growth factor β superfamily, Wnts, and fibroblast growth factors (FGF) all play a role in differentiation of ESCs into cardiomyocytes [63]. The delicate balance needed for sufficient qualitative and quantitative differentiation into cardiomyocytes is a major barrier for the use of these proteins as a therapy for cardiac regeneration. Furthermore, the effect of these proteins on resident cardiac stem cells is largely unknown, and it is unlikely that local or systemic administration of a single one of these proteins will induce significant differentiation of CSCs.

Future directions

The past two decades have been marked by two exciting scientific revolutions: one is the clinical application of protein therapeutics in many different diseases, and another is the emergence of regenerative biology and regenerative medicine. In the next decade, these two revolutions might merge to lead to the use of proteins for cardiac regeneration. Until now, the only protein with cardiac regenerative potential used in clinical studies after MI is G-CSF, but its true potential to induce formation of new myocytes or vessels is highly controversial [28, 64].

Clinical studies with transplantation of bone marrow stem cells after MI have shown a small but reproducible effect on cardiac function [3]. However, the underlying mechanism is probably not extensive differentiation into cardiomyocytes but paracrine effects on neighboring myocardium [3]. It is conceivable that proteins secreted by these cells could be identified, purified and used as a protein therapeutic. There is also a need for identification of

new proteins that attract stem cells to the damaged myocardium. SDF-1 and HGF are stem cell chemoattractants that may be useful in the myocardium.

Although the proteins described in this review have shown preclinical potential for treatment of myocardial infarction, the mechanistic understanding and the knowledge of the pharmacodynamic/pharmacokinetic profile is far too limited to truly label them as drug candidates. Furthermore, further research should not focus solely on regeneration of lost myocardium because some proteins – including Thymosin- β 4, Adiponectin, and IL-33 – improve cardiac function after MI, possibly by preventing cardiomyocyte death.

As our understanding of cardiac and stem cell biology increases, new proteins that promote cardiac regeneration will be discovered and existing proteins will be more extensively studied for effects on cardiac regeneration. Also, major biotechnological advances – e.g. glycosylation [5] or PEGylation [65] – have been made that allow for prolonged activity and better pharmacokinetic properties of proteins used as a drug. This will lead to more and better treatment options for patients suffering from MI and heart failure.

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Figure 1. Regeneration of the heart by 4 different classes of proteins

See text for details. A) FGF and VEGF increase angiogenesis. B) G-CSF mobilizes bone marrow hematopoietic stem cells and SDF-1 attracts endothelial progenitor cells. HGF attracts cardiac stem cells. C) Neuregulin and periostin can induce division of adult cardiomyocytes. D) IGF-1 induces maturation and differentiation of cardiac stem cells.

Table 1

Overview of preclinical studies using proteins for treatment of myocardial infarction.

Protein	Mode of action	Route	Animal model	Outcome (vs. Control)	Ref
HGF	Anti-apoptotic	IV	Rat	Infarct size -10.3% (P<0.01)	[31]
	Anti-apoptotic	IV	Mouse	EF +5.4% (P=0.02)	[33]
	Chemotactic on CSCs	IM	Mouse	Mortality -34% (P<0.0001)	[32]
	Angiogenic	IV	Mouse	EF +5.4% (P=0.02)	[33]
IL-11	Anti-apoptotic	IV	Mice	+dP/dt +359mmHg/s (P<0.05)	[66]
IL-33	Anti-apoptotic	SC	Rat	EF +9.6% (P<0.01)	[67]
Adiponectin	Anti-apoptotic	IC	Pig	+dP/dt +300mmHg/s (P<0.05)	[68]
Thymosin-β4	Anti-apoptotic	IM+IP	Mouse	EF +29.5% (P<0.0001)	[69]
SDF-1	EPC recruitment	IM	Mouse	FS +8% (P<0.05)	[70]
	EPC recruitment	IM	Mouse	EF +17.4% (P<0.0001)	[71]
	EPC recruitment	IM	Rat	EF +15.5% (P<0.01)	[41]
Periostin	Mitogenic	Gelfoam patch	Rat	EF +15.7% (P<0.01)	[47]
Neuregulin	Mitogenic	IP	Mice	EF +9.5% (P<0.01)	[48]
IGF-1	CSC growth	IM with CSCs	Rat	EF +8.4% (P<0.05)	[58]
PDGF-BB	Anti-apoptotic	IM	Rat	EF +16.2% (P<0.01)	[72]
PTH	Stem cell mobilization	SC	Mice	EF +16.9% (P<0.05)	[73]
G-CSF	Stem cell mobilization	SC	Mouse	Mortality -56% (P<0.001)	[24]
	Stem cell mobilization	SC	Rat	FS +3.4% (P<0.05)	[74]
	Anti-apoptotic	SC	Mouse	FS +12% (P<0.05)	[28]
	Anti-apoptotic, Angiogenic	SC	Pig	FS +5% (P<0.05)	[75]
EPO	Angiogenic	IP	Rat	+dP/dt +1500mmHg/s (P<0.01)	[76]
FGF	Angiogenic	IC	Canine	EF +28% (P<0.05)	[10]
	Angiogenic	IC	Pig	No functional improvement	[77]

CSCs= cardiac stem cells, EF= ejection fraction, EPC= endothelial progenitor cell, EPO= erythropoietin, FGF= fibroblast growth factor, FS= fractional shortening, G-CSF= granulocyte colony stimulating factor, HGF= Hepatocyte growth factor, IC= intracoronary, IGF-1= insulin like growth factor-1, IM= intramyocardial, IP= intraperitoneal, PDGF-BB= Platelet derived growth factor-BB, PTH= Parathyroid hormone, SDF-1= Stromal cell derived factor-1.

Table 2

Desired properties of proteins used as therapeutics for myocardial regeneration after MI.

<i>Clearly defined biological mechanism</i>
Chemotactic on stem cells
Mitogenic on cardiomyocytes
Growth factor
Angiogenic
<i>No toxic effect on remote tissues</i>
No toxicity at pharmacological concentrations
Selective for target cell or tissue
No nephrotoxicity (important for small proteins)
No stimulation of tumor growth
<i>Not immunogenic</i>
No induction of neutralizing antibodies
<i>Known pharmacokinetic properties</i>
Long systemic half life
Known volume of distribution
<i>Feasible methods of delivery</i>
Systemic vs. local
Delivery vehicle