Protein promise in heart disease

Protein therapies are usually associated with anticancer regimens, however some initial clinical trials indicate that they could play a significant role in treating cardiovascular disease, based on their angiogenic properties. **Dr Thomas J Stegmann** explains

KEYWORDS: Cardiovascular disease (CVD); Protein therapy; Angiogenesis

Cardiovascular disease is the number one killer in the US, claiming around 2,500 lives a day, or one death every 35 seconds, according to the American Heart Association. This is more than the next four leading causes of death combined – cancer, chronic lower respiratory diseases, accidents and diabetes mellitus. And although there are several treatment options across the clinical spectrum of cardiovascular disease, a significant proportion of patients are unsuitable for many of the regimens on offer (see Box below).[1–3]

To combat this unmet need, protein therapy is being explored as a potential treatment. Although most of the 866 ongoing US FDA-authorised studies investigating protein therapy are cancer trials, 29 studies are focusing on protein therapy for cardiovascular disease (CVD). So far, the results look promising, and this article considers why this approach could be so valuable.

Magic molecules

Proteins are the most important molecules inside every living cell, tissue and organ, and perform all functions necessary for life: they regulate all our physiological reactions, they metabolise carbohydrates and fats, they defend our bodies against bacteria and viruses, they work as enzymes, exquisitely potent hormones, antibodies, cytokines and signalling peptides that transmit information into cells – they do everything.

Most human diseases are related to the malfunctioning of particular proteins, either systemically or locally. Today, a variety of human proteins can be produced with relative ease by means of genetic engineering and recombinant DNA technology. Large quantities of the desired protein can be made in mammalian cells, yeast or bacteria. Novel protein expression systems, including the newly introduced phage technology process, allow biologically active, properly folded, engineered

Heart disease: Prevalence, socio-economic impact and treatment

The clinical spectrum of cardiovascular disease comprises: coronary heart disease (CHD), congenital cardiovascular defects, heart failure, stroke, high blood pressure and peripheral arterial disease. Of the 71.3 million American adults with one or more types of CVD, 27.4 million are estimated to be aged 65 or older.

Preliminary mortality data show that CVD was the underlying cause of death in 37.3% of all deaths, or one of every 2.7, in the US in 2003. CVD as an underlying or contributing cause of death (1.4 million deaths) made up 58% of deaths in 2002. The estimated direct and indirect cost of CVD for 2006 is US$403.1 billion.[1]

In Europe, the numbers are similar: CVD causes nearly half of all deaths in Europe (49%) and in the EU (42%).[2] Each year, CVD causes more than 4.35 million deaths in Europe and over 1.9 million deaths in the EU. CVD is the main cause of death in women in all countries of Europe and is the main cause of death in men in all countries except France and San Marino. CHD by itself is the single most common cause of death in Europe, accounting for 1.95 million deaths a year. Overall, CVD is estimated to cost the EU economy US$169 billion a year.[2]

Methods of treating patients with severe medically refractory CHD include various catheter-based techniques (percutaneous coronary intervention or PCI) and such modifications as laser angioplasty, rotablator techniques and stent implantation; also surgical procedures, particularly coronary artery bypass grafting (CABG).

An estimated 664,000 PCI procedures on 652,000 patients and 467,000 CABG operations on 268,000 patients were performed in 2003 in the US.[1]

A significant number (estimated at 150,000 a year) of these patients, however, present with diffuse coronary artery disease, proximal stenosis and additional peripheral stenoses/occlusions of the coronary artery branches, small vessels, or severe general co-morbidities, which makes them unsuitable both for PCI and for CABG ('no-option' heart patients).

Owing to hereditary factors, Asian Indians in particular show this unfavourable pattern of CHD.

proteins to be manufactured and purified in high yield at a very reasonable cost.

Therapeutic proteins can be used as highly effective medical treatments – protein therapy – for a wide array of diseases in which the protein is either lacking or deficient (growth hormone and insulin), or the therapeutic protein is used to inhibit a biological process (antibodies that block blood supply to tumours).

A range of proteins and protein-based therapies are accepted treatments and have been introduced into the market: examples are Interleukin-2 for the treatment of HIV infection and advanced tumour stages; Interferon (IFN) alfa-2b and IFN alfa-2a (today most commonly used as pegylated form) for the treatment of hepatitis B and C; granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-GSF) for the treatment of HIV infection, haematological diseases and cancer; mycophenol for the treatment of chronic organ rejection and immunosuppression, or tumour necrosis factor (TNF) inhibitors for the treatment of rheumatoid arthritis. However, as yet no angiogenic protein-based therapy has been approved by the US FDA for the treatment of CVD.

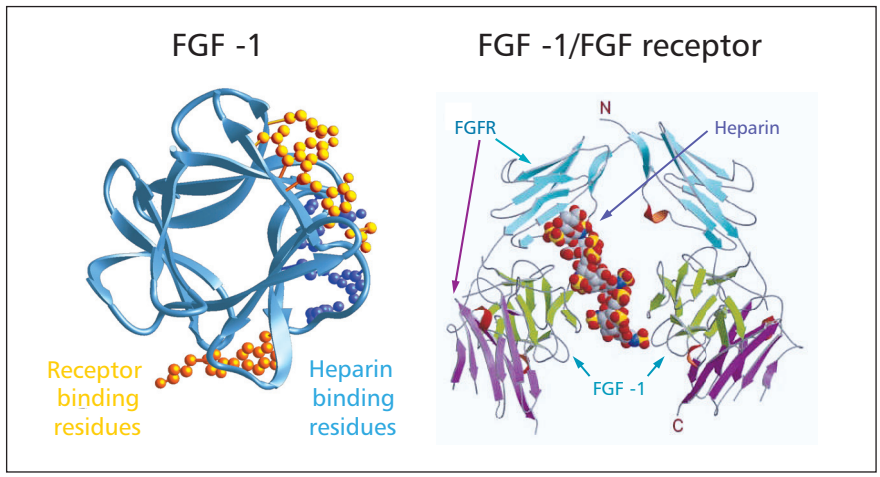

Figure 1: Three-dimensional structures of fibroblast growth factor 1 (FGF-1) and FGF-1 receptor (FGFR). The receptor binding sites are indicated in yellow, the heparin binding sites in blue.

Angiogenesis and CVD

To understand the implications of protein therapy in CVD, it is worth looking at the process of angiogenesis (the formation of new blood vessels) and its role in both disease and potential treatment. Research into angiogenesis started nearly 30 years ago and focused mainly on the inhibition of angiogenesis to limit tumour growth. In the 1980s, the isolation, characterisation and purification of the first angiogenic growth factors were reported; subsequently, inhibitors of angiogenesis were developed. Examples include angiostatin, endostatin, and tumstatin. In 2004, the US FDA approved the first anti-angiogenic protein therapy for cancer, Avastin (bevacizumab, rhuMAb-VEGF) to treat metastatic colorectal cancer in combination with established chemotherapy.

Research into angiogenesis and heart disease established that in blood vessels of mature organisms, the endothelial cells remain in a quiescent, non-proliferate state until they are stimulated by conditions, such as wounding, inflammation, hypoxia or ischaemia. The development of new vessels can be considered as the result of several processes:

- Dissolution of the matrix underlying the endothelial cell layer
- Migration, adhesion and proliferation of endothelial cells
- Formation of a new three-dimensional tube, which then lengthens from its tip as circulation is re-established
- In larger vessels, vascular smooth muscle cells also migrate and adhere to the newly deposited matrix of the nascent vessel.

Angiogenic growth factors (proteins) induce, promote and/or interfere with all these steps of angiogenesis.

A large variety of very potent proteins and polypeptides, termed 'growth factors', exists in the human body. Growth factors are naturally occurring proteins capable of stimulating cellular proliferation, maturation, and differentiation; they typically act as signalling molecules between cells and bind to specific receptors on the surface of their target cells. The best-known growth factors with proven angiogenic potency are fibroblast growth factor-1 (FGF-1) and vascular endothelial growth factor-1 or -A (VEGF-1). FGF-1 stimulates the proliferation and differentiation of all cell types necessary for building an arterial vessel – endothelial cells, smooth muscle cells, and fibroblasts (adventitial cells) – whereas VEGF-1 mainly stimulates endothelial cells, and increases the permeability of the vessel wall.

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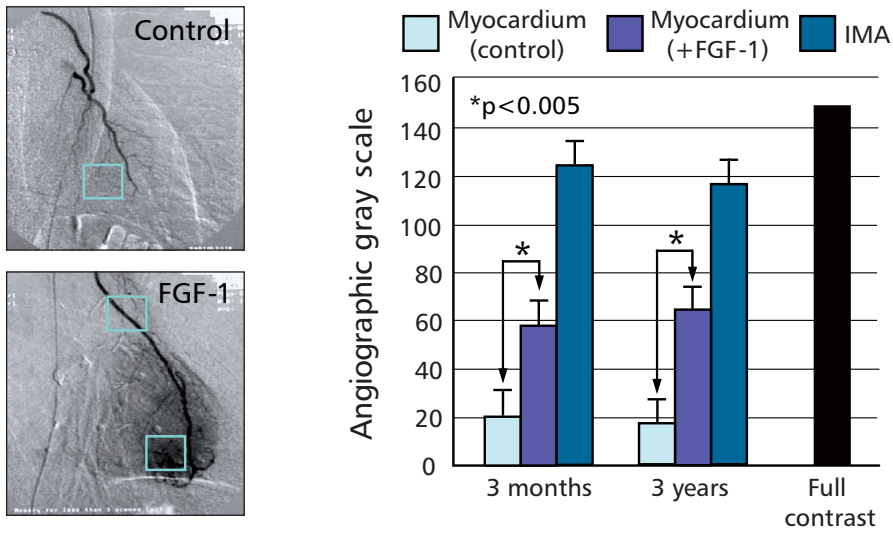
Developing protein therapies

In contrast to the still very active research and development of anti-angiogenic proteins for treatment of malignant tumours, research into the use of angiogenic proteins in promoting angiogenesis as a therapeutic option for CVD is still at



Left: Coronary angiography indicating an increased capillary density after FGF-1 treatment in the LAD territory (FGF-1) compared with the control. Right: Semi-quantitative measurement of pixel density ('grey value') in angiograms, indicating a threefold increase of vessel density in the treated humans (myocardium + FGF-1) after three months and three years. (LAD = Left anterior descending coronary artery; IMA = Internal mammary artery.)

Figure 2: Results from the first human clinical trial.[4,5]

an early stage – as reflected by the accordingly small number of FDA-authorised clinical trials. In light of the importance, frequency and socio-economic impact of CVD in the Western world, however, and due to the limitations of conventional treatment modalities (PCI, CABG), especially for diffuse atherosclerotic diseases, the introduction of protein therapy as pro-angiogenic treatment could become the next important step in fighting CVD.[3]

In 1998, we introduced, for the first time in humans, protein therapy into the therapeutic arsenal of treatment modalities for CHD.[4,5] Because of the potency of FGF-1 (acidic FGF) in promoting angiogenesis with respect to proliferation of all cell types needed for vascular growth, FGF-1 was used as single protein therapy for patients suffering from diffuse CHD. In the context of this first-in-man clinical study, it should be stressed that only the intramyocardial application of the growth factor appeared to be effective. This has the attractive feature of avoiding a systemic distribution and circulation of the protein, which would have potentially undesirable side-effects.[4]

The FGF family, with its prototype members FGF-1 and FGF-2 (basic FGF), consists of at least 22 known members to date. They all are 16–18 kDa single chain peptides and display high affinity to heparin and heparin sulfate. FGFs stimulate a variety of cellular functions by binding to cell surface FGF-receptors (FGFR) in the presence of heparin proteoglycans (see Figure 1). FGFRs are single chain receptor tyrosine kinases that become activated through autophosphorylation induced by a mechanism of FGF mediated receptor dimerisation. Receptor activation gives rise to a signal transduction cascade that leads to gene activation and diverse biological responses, including cell differentiation, proliferation and matrix dissolution, thus initiating a process of mitogenic activity critical for the growth of endothelial cells, fibroblasts, and smooth muscle cells.

These biological effects of FGF are established as a result of intracellular signal transduction initiated by the growth factor binding to and activating its receptors. The structure of the FGFRs includes an extracellular portion containing the ligand-binding domain, a transmembrane segment and a tyrosine kinase domain in the cytoplasm. FGF-1 is a monomeric single chain polypeptide consisting of 141 amino acids, and it binds to the second and third immunoglobulin like domains of all four FGFRs. FGF-1 contains no secretory leader sequence and is stabilised by binding to heparin.[6]

With patient safety being the first consideration, the first clinical trial was performed in 40 patients, who needed CABG primarily; additional diffuse CHD was simultaneously treated by local injection of FGF-1 intramyocardially as adjunct to bypass surgery (20 patients). The control group (20 patients) received heat-denatured FGF-1. Figure 2 summarises the three-month and three-year results. No side-effects were seen and a threefold increase

The introduction of protein therapy as pro-angiogenic treatment could become the next important step in fighting cardiovascular disease

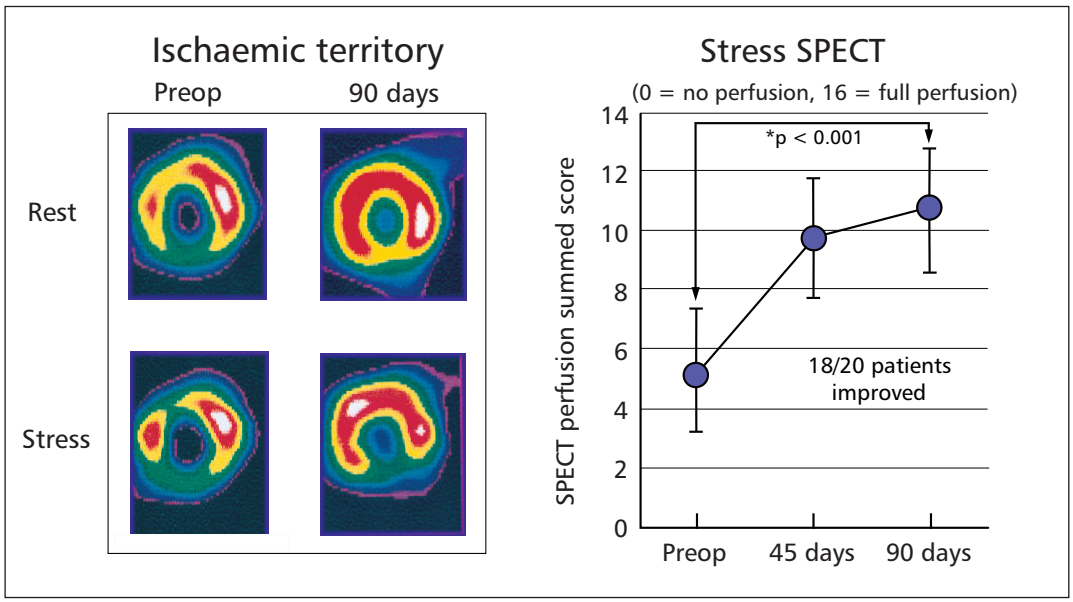


Figure 3: ^{99m}Tc-sestamibi stress SPECT imaging of the left ventricle preoperatively and 90 days after FGF-1 treatment as sole therapy for no-option heart patients.[7] Significant post-treatment improvement of myocardial perfusion. (SPECT = Single photon emission computed tomography.)

in vascular density was observed within the treated myocardium group compared with the control group, over both the three-month and three-year periods. There was a strictly localised angiogenic effect in the treated myocardium; the angiogenic response to FGF-1 protein delivery in the myocardium persisted over years; there was also an improvement of the myocardium regarding perfusion and function.[5]

In a second clinical trial that included 20 patients with CHD and no option for PCI and/or CABG, the FGF-1 administration as sole therapy was accomplished through mini-thoracotomy.[7] That second study, completed in 2000, focused on both safety and the functional status of the patients after therapy. The results were as follows: improvement of myocardial perfusion demonstrated by SPECT scans, under rest as well as under stress conditions (see Figure 3); improvement of maximum working capacity in 18 patients; improvement in CCS-classification of at least one class in all patients. Since then, an FDA-authorised Phase I human clinical trial has been successfully performed in the US, confirming the results of the two German trials.[8]

This new type of treatment for severe CVD – using a human protein with no immunogenic response, no need for viral vectors, localised effect at the target tissue and predictability of dose – is just one example of the promise of protein therapy. If the CVD study results can be reproduced in other therapeutic domains, then protein therapy could well become the treatment modality of choice for many disorders. GCPj

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