Heart failure: running to the rescue

Kathryn Senior, Freelance writer

A recent preliminary study suggests that gene transfer could provide a much-needed therapy for a common and currently untreatable form of heart failure. Joseph Metzger (University of Michigan Medical School, Ann Arbor, MI, USA) and a 15-strong team of collaborators have shown that expressing parvalbumin in ventricular muscle can reverse the symptoms of diastolic heart failure in a rat model of the disease[1]. 'Showing that a gene can be transferred into the intact heart and have functional consequences is a considerable achievement,' says Phillip Poole-Wilson, Professor of Cardiology at the National Heart and Lung Institute, Imperial College (London, UK).

Heart failure is a major health burden in the developed world: in the USA, for example, 5 million people are affected and there are 700,000 new cases each year. Systolic heart failure accounts for 60% of cases and can be treated by drugs such as β-blockers. However, no drugs have yet been developed to directly correct diastolic heart failure, which accounts for the remaining 40% of cases. 'This form of the disease occurs when heart muscle takes too long to relax between contractions and frequently leads to congestive heart failure and a significant reduction in quality of life,' explains Metzger.

Pathophysiology of the disease

In the failing heart, degeneration reduces the capacity of the contractile mechanism in the ventricular and atrial muscle to normalize calcium ion levels in time for the next contraction. In a healthy heart, the sarcoplasmic reticulum (SR) releases a rush of calcium ions to activate contraction in the cell. This reveals myosin-binding sites in actin filaments in the fibre. Myosin then attaches, and, using energy from ATP hydrolysis, pulls the actin filament along, causing the muscle fibre to contract. As calcium ions are reabsorbed into the SR, the myosin heads detach and the muscle relaxes. This process of contraction is essentially the same in cardiac and skeletal muscle.

However, in fast-twitch muscle fibres (such as those that are found in the leg muscles of an active runner), the process of relaxation is speeded up because parvalbumin, a calcium-binding protein that occurs naturally in these specialized skeletal muscle fibres, binds calcium ions, reducing their concentration very quickly. 'Although parvalbumin does not occur naturally in heart tissue, it provides an attractive molecule to solve the problems of diastolic heart failure by sequestering calcium ions and enabling the heart muscle to relax normally between beats,' says Metzger.

Effects of parvalbumin

In 1999, Metzger's group demonstrated that the relaxation of isolated adult human cardiac myocytes cultured *in vitro* could be accelerated by parvalbumin expression[2]. Their current study is the first to investigate the effect of parvalbumin expression *in vivo*. The group showed that gene transfer of the human full-length α-parvalbumin cDNA directly into the muscle of the left ventricle resulted in physiologically relevant levels of parvalbumin six days after gene transfer. Further experiments showed that the expressed parvalbumin specifically accelerated the mechanical relaxation properties of the intact myocardium under physiological pacing conditions.

The most exciting result came when the group transferred the parvalbumin gene into the hearts of rats with hypothyroidism. 'Although it is widely acknowledged that there is no perfect animal model for human diastolic heart failure, the hypothyroid rat model does mimic the human disease in terms of reduced calcium sequestration rate and slowed myocardial relaxation', says Metzger. In the model, parvalbumin expression restored the *in vivo* relaxation performance of hypothyroid rats and the animals showed normal diastolic function after treatment.

Issues to be resolved

Metzger stresses that many issues need to be addressed before gene transfer with parvalbumin can reach clinical trials. 'In addition to the general concerns

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surrounding the safety of gene therapy, it is also important to develop a suitable and convenient delivery system,' he says. Catheter delivery is one option, targeted delivery by identifying key markers on target cells is another. Currently, in addition to investigating possible vectors that could be used to replace direct injection, Metzger and his team are developing transgenic mice that express parvalbumin in heart muscle to carry out dose–response studies. 'This will enable us to investigate what side-effects might result from over-expression,' says Metzger.

Poole-Wilson also warns that it will be important to determine whether the use of parvalbumin is energy consuming. 'Once the calcium has been attached to parvalbumin, it must then be released and either temporarily taken up by the SR or excreted by the cell. Both these processes consume energy and it will be interesting to see how much energy is required in the human heart, which has a slightly different calcium release system compared with the rat heart,' he says. Metzger agrees that energetics is an important issue, 'particularly because the failing heart is thought to be energetically compromised.'

Metzger and colleagues are also moving into larger mammalian models; studies of gene transfer in dogs are under way, and *in vitro* studies using myocytes from heart failure patients who undergo heart replacement are also planned. If a safe, high-efficiency vector can be found, and if stable and long-lived expression of parvalbumin can be achieved, human trials could be possible, but this could be a way off yet. Poole-Wilson agrees but emphasizes that this study is an impressive proof-of-principle that could have substantial application. 'There is currently a race between the clinical application of pumping devices, cell transplantation and gene therapy. Seeing how these three approaches to heart failure will fit into the current therapeutic armatarium should be interesting,' he concludes.

References

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Vaccine stops blood supply

Sharon Dorrell, Freelance writer

Researchers at EntreMed (Rockville, MD, USA) believe they can arrest tumour growth with an experimental vaccine designed to block angiogenesis, the process by which tumours create their own blood supply (see Box 1). Stacy Plum and colleagues vaccinated mice with a 42-amino acid peptide derived from basic fibroblast growth factor (FGF-2)[1], one of the factors that stimulates angiogenesis[1]. Vaccination inhibited new blood vessel formation and protected mice against experimental melanoma and lung carcinoma metastasis (Fig. 1).

Several approaches to blocking angiogenesis are currently under investigation. These include inhibitors of vascular endothelial growth factors (VEGFs) and inhibitors of the degradative enzymes involved in the early stages of angiogenesis[2]. However, the scientists at Entremed believe their technique is unique. Anne Fortier (Senior Director, Preclinical Sciences, EntreMed) believes that no one has yet attempted a vaccine approach that targets factors other than cancer antigens. 'This approach will enable us to target both processes: prevention of new tumour development and

Box 1. Angiogenesis

Angiogenesis is key to the success of the invasion of the body by tumours. Without an adequate blood supply, tumours cannot grow beyond a few millimetres in size and might only survive as thin layers of cells that cause no symptoms[2,3]. A rich blood supply, however, enables them to grow and shed their cells into the bloodstream, thus giving them access to other tissues where secondary tumours can form.

The process begins with the stimulation of vascular endothelial cells to degrade the local basement membrane and migrate to form new capillary branches from the parent blood vessel[3]. This process requires several degradative enzymes as well as vascular and fibroblast growth factors to build the new blood vessels. These factors include FGF-2, other fibroblast growth factors, vascular endothelial growth factors, tumour necrosis factor-α, platelet-derived growth factor, interleukin-8, as well as other peptide and non-peptide factors[2]. By inhibiting this process and cutting off tumour blood supply, it is possible to kill tumour cells and arrest tumour growth and spread[2].

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