Ischaemic injuries

Professor Derek Yellon and **Dr Sean Davidson** discuss their research on preventing injury to the heart from ischaemia-reperfusion and the potentially protective role of exosomes

Could you begin by describing your primary research interests?

DY: I mainly study the pathophysiology of ischaemia-reperfusion and acute myocardial infarction, in both the basic and clinical setting. This involves investigating both ways to protect the heart and the molecular and cellular aspects of adaptation to ischaemic injury.

SD: My main research area overlaps with Yellon's, being focused on the molecular aspects of cellular injury with specific attention to nanoparticles (exosomes) and their role in the cardiovascular system. I also specialise in using optical imaging to visualise cellular responses to injury and delineate the mechanisms involved in remote ischaemic conditioning (RIC).

How did you come to develop an interest in RIC?

DY&SD: Myocardial preconditioning was discovered in 1986 by serendipity when investigators at Duke University, USA, demonstrated that short periods of loss and return of coronary blood flow could significantly protect the heart from a subsequent ischaemic insult. This phenomenon was shown to be the most powerful form of cardiac protection known to date and attracted leading investigators around the world (including ourselves) who wanted to elucidate its mechanisms and potential clinical significance.

Importantly, we were the first to demonstrate that a non-invasive RIC method could protect the hearts of patients undergoing routine coronary artery bypass graft (CABG). We are pleased to state that we have now been given significant funding by the Medical Research Council (MRC), the National Institute for Health Research (NIHR) and the British Heart Foundation (BHF) to conduct the first multi-centre outcome study to test the efficacy of this method in patients undergoing CABG.

What brought the potential cardioprotective role of exosomes to your attention?

DY: Exosomes are nanoscale lipid vesicles that are released from multi-vesicular bodies in cells and are believed, under appropriate conditions, to act as carriers of protective proteins. Davidson came across their importance when, while trying to understand how messages could be transported from the RIC limb to the heart (or other organ under threat of injury), he learned of their ability to transport multiple proteins while evading the immune system. We immediately saw the potential that these nanoparticles may have as delivery vectors and undertook initial studies to investigate their ability to protect the heart from ischaemia-reperfusion injury.

When studying exosomes, what challenges have you faced?

DY&SD: The first challenge we have faced was, simply, how to measure them; this is difficult as they are smaller than the wavelength of light and therefore can't be seen using standard techniques. However, we became aware of recently developed specialised equipment that enables the detection of these nanoparticles using a technique involving laser diffraction. We immediately raised the funds to purchase this equipment, which allowed us to directly demonstrate that RIC stimulates the release of exosomes in animals and humans.

Do researchers at the Hatter Cardiovascular Institute at University College London (UCL), where you are based, collaborate with other research groups?

DY&SD: We have established a number of productive collaborations. Within UCL, we are working closely with Professor Steve Humphries and Dr Natassa Kalea to investigate the ability of exosomes to deliver microRNA and reprogram cells. We are also investigating the cellular mitochondrial response, using multi-photon imaging, together with Professor Micheal Duchen.

Internationally, we are working closely with Professor Jim Downey from the University of South Alabama, USA, investigating mitochondrial DNA and its detrimental role in inflammatory response. We are also collaborating with Dr Borja Ibanez from the Spanish National Centre for Cardiovascular Research in Madrid, whose large animal facilities will potentially enable us to translate our studies to a clinically relevant model.

Which of your professional achievements would you consider your greatest to date?

DY: My greatest professional achievement has been setting up the Hatter Cardiovascular Institute at UCL. What started out as a relatively small group of enthusiastic basic and clinical scientists has developed into an internationally recognised cardiovascular institute that houses approximately 50 scientists, clinicians and support staff, and is funded by major grants from the MRC, BHF, Wellcome Trust, NIHR Biomedical Research Centre, The Hatter Foundation and the Rosetrees Trust. The Institute's ethos is very much focused on translational research related to whether it is possible to protect the heart during an acute myocardial infarction; we take treatments from the experimental basic science stage in our laboratories through to patients in the clinic. We are fortunate to have some of the world's very best basic scientists, as well as a range of world-class clinical cardiovascular services through which to recruit patients for trials.

Natural heart protection

The Hatter Cardiovascular Institute is conducting the first multi-centre outcome trial of a simple, safe and non-invasive procedure to protect the heart from injury during coronary artery bypass surgery or heart attack, while investigating the cellular communication mechanisms that underlie its effectiveness

CORONARY HEART DISEASE, in which the arteries and small vessels that transport blood and oxygen to the heart become narrowed by fatty deposits of plaque building up along their walls, is currently the leading cause of death in the UK. Furthermore, as populations age and the incidence of risk factors such as obesity and diabetes continues to rise, it is predicted that the number of deaths caused by coronary heart disease will increase along with them. Of these deaths, the majority are caused by heart attacks.

Successful treatment for heart attack and during cardiac surgery, relies on the restoration of blood flow (reperfusion) of heart tissue. Ironically, however, the reperfusion procedure exacerbates the damage caused by ischaemia, which can be lethal to cells in the myocardium. Ischaemia and reperfusion injury thus bring about metabolic and structural changes to the heart, which can result in abnormal heart muscle function and lead to heart failure.

REMOTE POSSIBILITIES

For years it has been known that ischaemic preconditioning – a short reiteration of withdrawal and re-supply of blood to the heart – can protect against heart attack. However, as an invasive procedure, its use in heart surgery was limited. More recently, it was found that preconditioning applied to other tissues or visceral organs remote from the heart, such as the kidney, have equivalently protective effects for the heart, although this form of so-called remote ischaemic conditioning (RIC) was also invasive and so attracted few adherents among cardiologists and cardiac surgeons. According to Professor Derek Yellon, founder and Director of the Hatter Cardiovascular Institute at University College London (UCL), it was only recently that a non-invasive method of RIC was discovered and the prospect of its utilisation to routinely protect the heart from cell death during myocardial infarction or surgery became real: "Collaborators of ours at UCL found that a blood pressure cuff, placed on the upper arm or lower limb and inflated and deflated three or four times, resulted in a signal being sent to the heart to protect it during an ischaemic insult".

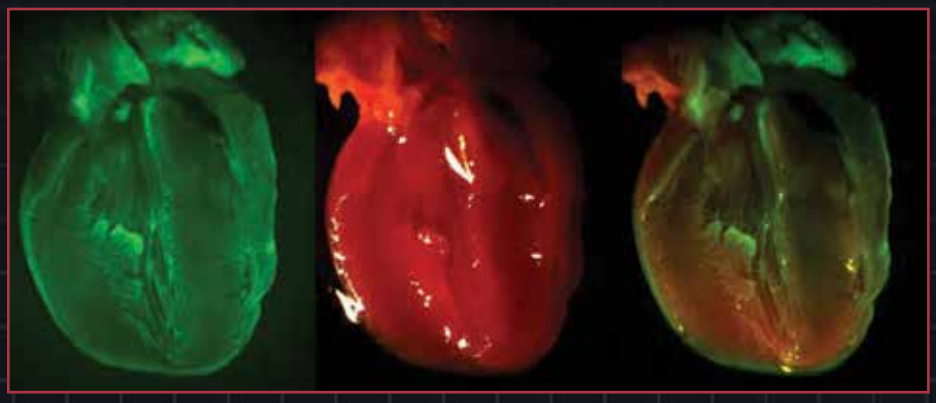
Since then, the effects of this low-cost, non-invasive method of RIC have been explored in a number of studies using patients undergoing surgical procedures including coronary artery bypass graft (CABG), percutaneous coronary intervention and kidney transplantation; indeed, Yellon and his team at the Institute conducted the first proof-of-concept clinical study in patients undergoing scheduled CABG. Now, following on from findings that indicate non-invasive RIC may offer significant benefits in terms of one-year post-operative survival of CABG, Yellon and colleagues are running the first large-scale trial of the approach in a study entitled 'Effect of Remote Ischaemic preConditioning on clinical outcomes in patients undergoing Coronary Artery Bypass Graft surgery' (ERICCA).

ERICCA

The study is a multi-centre double blind trial of the effect of RIC on patient outcomes after receiving CABG with or without accompanying heart valve surgery. Heart valve surgery patients have been specifically included as, although typically higher risk, earlier proof-of-concept trials have clearly suggested that RIC may have a beneficial influence on the outcomes for such patients.

1,610 adult patients calculated to be at high risk of death following heart surgery have been recruited for the study from 27 UK tertiary cardiac centres, with each being randomly given either a functional or dummy RIC procedure on the day of their operation. In all cases, four cycles of blood pressure cuff inflation/deflation are applied to the patient's arm or leg after anaesthesia, prior to reperfusion and surgery. The research nurse undertaking this procedure is the only individual present in the cardiac centre who knows whether the cuff is operational or not, with all subsequent follow-ups, biomarker measurement and data collection being carried out by other staff.

The principal aim of ERICCA is to establish whether RIC improves patient outcomes in terms of subsequent fatality from cardiovascular causes, non-fatal heart attack or stroke, or the need for revascularisation, within both 30 days and one year of surgery. ERICCA will also assess whether RIC reduces the extent of complications after surgery in terms of heart damage, maintenance of left ventricle systolic function and the development of acute kidney injury. Other measures will include RIC's impact on the patient's length of stay in intensive care and in hospital following the operation; whether post-operative fatalities from any cause are reduced; and whether there is any reduction in requirements for inotropic drugs to boost heart muscle contraction ability. Finally, ERICCA will



A transgenic mouse heart glows green in response to calcium, enabling the team to visualise the response to ischaemia.


A multi-photon image of cells within a live heart.

carry out assessments of exercise tolerance (via a six minute walking test) and overall sense of quality of life on recruitment, six weeks after treatment, and then quarterly.

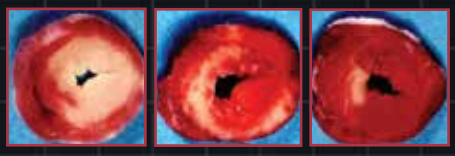
TRACKING SIGNALLING

So far, animal and clinical trials have provided compelling evidence of the benefits of non-invasive RIC. However, how the signal reaches the major organs from the limb that receives RIC and what mechanisms confer protection to the heart remain unclear. One proposition is that a messenger molecule carried in the blood is the source; another is that the mechanism employs as yet undefined neural pathways.

Yellon's colleague Dr Sean Davidson, a Senior Research Fellow and Associate Director at the Hatter Cardiovascular Institute, was elected to investigate in detail the part played by mitochondria – cellular energy powerhouses fundamental to cell activity and survival – in this process. "Our team was instrumental in investigating the basic pro-survival signalling mechanisms involved in protection by recognising that the pathway converged on mitochondria," recalls Davidson. "More specifically, Yellon identified the pro-survival kinases, coining them the 'reperfusion injury salvage kinase (RISK)' pathway." Building upon this discovery, Yellon and Davidson went on to explore the relationship between mitochondria and the RISK pathway, and demonstrated that the permeability transition pore (PTP) of the inner mitochondrial membrane was directly involved in preconditioning. This has led them to propose that the PTP acts as the end-effector in the protection process, and infer that the RISK pathway and inhibition of PTP opening might serve as therapeutic targets in cardiac protection strategies to improve prognoses for acute heart attack patients.

Using specialised medical imaging techniques, Yellon and Davidson are currently exploring the role of exosomes in mitochondrial protection against heart cell death. Exosomes are microvesicles measuring 30-100 nanometres in diameter and, present in most bodily fluids such as blood, saliva and urine, are thought to ferry proteins, messenger RNA and microRNA while protecting their cargo from the immune system and enzymatic degradation. The UCL researchers are testing the possibility that the transfer of exosomes and their cargo to cells may actually be core to intercellular communication, and so underlie the process of signalling from the limb to which non-invasive RIC is applied to the visceral organs. So far, in animal studies, the investigators have found that exosomes are highly protective of the heart, activating the same protective kinase pathways used in preconditioning. Intriguingly, it appears that the mechanism may be evolutionarily conserved across species.

Yellon and Davidson's objective is ultimately to exploit the innate survival mechanisms that prevent cell death and so myocardial injury from ischaemia-reperfusion. In this, they see clear potential for harnessing the abilities of exosomes by manipulating them to deliver specific proteins or by stimulating the production and transport of pro-survival exosomes in the body. "We are now learning what causes the release of exosomes from cells, what regulates their content, and how to exploit the proteins they carry to the advantage of cells under threat of injury," Yellon enthuses. They also hope to realise the apparent potential of exosomes to exert protective effects on the heart in patients with co-morbidities such as diabetes to improve outcomes.

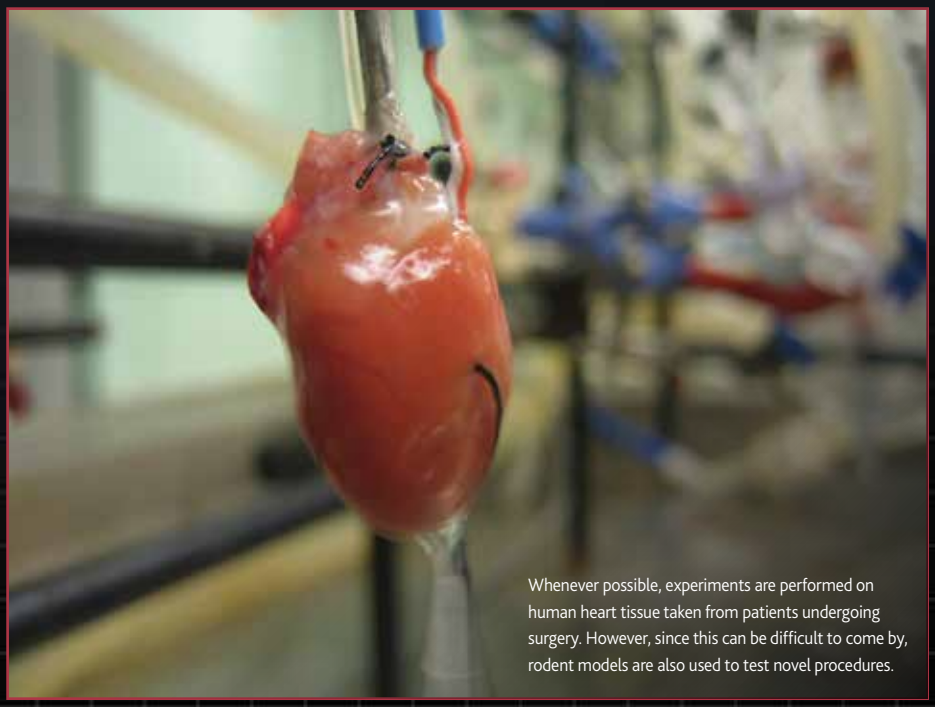

The white in these tissue cross-sections shows the extent of damage caused to the heart by myocardial infarction. Without protection (**left**), the damage is extensive. With preconditioning (**centre**) and RIC (**right**), damage is significantly reduced.

THE HEART OF THE MATTER

In addition to these avenues of investigation, the Hatter Cardiovascular Institute is also carrying out two further studies related to non-invasive RIC. In light of the fact that certain biochemical changes and aspects of mitochondrial regulation play fundamental roles in the orchestration of cell death, the first project (with support from the UK Medical Research Council and the British Heart Foundation) is utilising multi-photon imaging to examine the response of myocardial calcium and mitochondria to ischaemia-reperfusion injury. "This allows us to directly visualise what is happening inside the live heart," explains Davidson.

In the second project, investigators are also analysing the importance of the cytokine stromal cell-derived factor 1α (SDF-1α) messenger molecule in the process of heart protection during ischaemia and reperfusion. "This cytokine has dual activities, being an important regulator of stem cell homing to sites of myocardial injury, in addition to conferring direct protective effects in the setting of acute myocardial infarction," Yellon elaborates. Having recently published an initial study suggesting that SDF-1α may play a role in RIC, Yellon and Davidson are now investigating whether SDF-1α is also carried by exosomes.

Interest in the possibilities presented by RIC is now growing rapidly on a global scale, with an ever-increasing number of research groups attempting to test both the efficacy of the procedure and elucidate the mechanisms that underlie it. "A race is on around the world to try and be the first to identify the factor that signals from the limb to the heart," reveal Yellon and Davidson. Whether or not the Hatter Cardiovascular Institute will 'win' this race remains to be seen; either way, it is certain humanity as a whole will benefit from the pioneering research they are conducting in this area.


Whenever possible, experiments are performed on human heart tissue taken from patients undergoing surgery. However, since this can be difficult to come by, rodent models are also used to test novel procedures.

CORONARY HEART DISEASE IS THE SINGLE LARGEST CAUSE OF DEATH IN THE UK

IT KILLS AROUND 1 IN 6 MEN AND 1 IN 10 WOMEN

SOMEONE DIES FROM A HEART ATTACK IN THE UK EVERY 7 MINUTES

Source: The British Heart Foundation

INTELLIGENCE

ERICCA

EFFECT OF REMOTE ISCHAEMIC PRECONDITIONING ON CLINICAL OUTCOMES IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFT SURGERY

OBJECTIVES

To determine whether remote ischaemic preconditioning improves clinical outcomes after cardiac surgery.

KEY COLLABORATOR

Dr Derek Hausenloy, Hatter Cardiovascular Institute at University College London

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SEAN DAVIDSON obtained his PhD in Melbourne, Australia, on methods of protecting cells from heat stress. He undertook postdoctoral work at the École Normale Supérieure in Paris, then moved to London in 1998 and is now Senior Research Fellow and Associate Director at the Hatter Cardiovascular Institute at UCL, where his focus is on developing methods to protect the heart from another type of stress – namely, ischaemia and reperfusion injury.