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REPORT

Trials, tribulations and speculation! Report from the 7th Biennial Hatter Cardiovascular Institute Workshop

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Introduction

The 7th biennial Hatter Cardiovascular Institute Workshop, comprising 21 leading basic science and clinical experts, was held in South Africa in August 2012 to discuss the current cutting edge status of cardioprotection and the application of cardioprotective modalities in the clinical management of myocardial ischaemia/reperfusion injury in the context of acute coronary syndromes and cardiac surgery. The meeting, chaired by Professor Derek Yellon and Professor Lionel Opie, was run to a format of previous Hatter Cardiovascular workshops with data presented by

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proponents followed by discussion and debate by the faculty.

Novel therapeutic targets

Microparticles in cardioprotection

Sean Davidson and Derek Yellon described their recent foray into the burgeoning field of endogenous nanoparticles. These particles, exosomes and microparticles, are released into the blood from multiple cell types including activated platelets and epithelial and endothelial cells. They are thought to carry microRNA and mRNA with the potential for genetic exchange between cell types, as well as transport of signalosomes and even cell-surface receptors [2], all of which have the potential for delivering signals to the myocardium that modulate inflammation, angiogenesis or cardioprotection. They presented data confirming and quantifying the presence of these particles in rat and human plasma, and showed that administration of purified microparticles leads to attenuation of infarct size when administered to Langendorff-perfused rat heart and reduced cell death in cardiac-derived HL-1 cells subjected to simulated ischaemia/reperfusion. Fluorescently labelled microparticles also show increased incorporation into HL-1 cells. Intriguingly, they also demonstrated that the number of microparticles can be altered under certain conditions or by certain treatments, leading to their proposal that it may be possible to harness them for clinical purposes.

This preliminary work was considered very interesting, although a number of questions remained, particularly as to the parent cell type of the microparticles released by remote conditioning and the efficacy of uptake within the myocardium. Moreover, the mechanism of protection still remains unclear, but this is the subject of on-going study.

Mitochondrial morphology

Derek Hausenloy and Derek Yellon presented the latest data looking at the role of mitochondrial morphology in the context of ischaemia/reperfusion injury, with the hypothesis that preventing mitochondrial fragmentation, or fission, during ischaemia/reperfusion injury would lead to a more cardioprotective phenotype [24]. In cardiac-derived HL-1 cells, promotion of mitochondrial fusion proteins such as Mfn-1 and Mfn-2, or using a dominant negative mutation of the mitochondrial fission protein, Drp1, promoted mitochondrial fusion and delayed the time to mitochondrial permeability transition pore (mPTP) opening in response to reactive oxygen species generating laser-light exposure in TMRM-loaded cells [25]. Similarly, a pharmacological inhibitor of Drp1 was found to be cardioprotective in the adult rat heart following ischaemia/reperfusion injury. Combined Mfn-1/Mfn-2 knockout adult hearts demonstrated increased mitochondrial fragmentation and the hearts demonstrated a dilated cardiomyopathy [25]. Data were also shown examining the role of DJ-1 in the heart. Mutations in this protein induce mitochondrial dysfunction and are responsible for a genetic form of Parkinson's disease. Data were shown that over-expressing this protein in a cardiac cell line induced mitochondrial elongation, delayed mPTP opening and reduced cell death following simulated ischaemia/reperfusion injury. In the adult, DJ-1 deficient hearts had greater mitochondrial fragmentation concomitant with increased susceptibility to ischaemia/reperfusion injury. Interestingly, these hearts were also partially resistant to the protection elicited by ischaemic preconditioning.

The data were found to be very interesting, although the link between mitochondrial fusion and mPTP function is currently unclear; there are technical challenges in terms of determining mitochondrial morphology in adult cardiac myocytes when compared to other cell types, given the highly structured nature of the myocyte and multiple mitochondrial populations (subsarcolemmal, interfibrillar and perinuclear [23]).

Platelets as a target for cardioprotection

Antiplatelet therapy in the form of $P2Y_{12}$ inhibitors such as clopidogrel and ticagrelor is an integral part of the immediate management of ACS. James Downey presented data that these agents have an additional pleiotropic effect on recruiting cardioprotective signalling that is dependent on the presence of platelets, but independent of their impact upon platelet function and aggregation. These drugs, including the intravenous $P2Y_{12}$ inhibitor, cangrelor, attenuate infarct size when administered to animals before reperfusion, a protective effect that depends on the activation of adenosine A_{2B} receptors and reperfusion injury salvage kinases (RISK), redox signalling, and opening of mitochondrial K_{ATP} (mK_{ATP}). Interestingly, protection from $P2Y_{12}$ inhibitors is not additive to that from ischaemic conditioning, thereby suggesting a conditioning-like protective mechanism. Given clinical data showing the genuine benefits of adding $P2Y_{12}$ inhibitors to aspirin and heparin (neither of which reduce infarct size) in the management of ACS, leads to the speculation that there may be a direct cardioprotective element to the benefits observed—above and beyond the anti-thrombotic effect for which these drugs were originally designed.

During the discussion of these data, it was observed that there has been a significant shift towards the adoption of various $P2Y_{12}$ inhibitors during the lifetime of many of the

clinical trials undertaken to study the efficacy of conditioning protocols. The recent retrospective analysis by Roubille et al. [30] reveals that loading with clopidogrel acted as a confounder in assessing the protection from ischaemic postconditioning in patients. This phenomenon deserves further investigation to see if all antiplatelet drugs are similarly protective as well as retrospective analysis of previous trial data to ascertain any impact of antiplatelet therapy on infarct limitation.

Matrix metalloproteinases and cardioprotection

Matrix metalloproteinases (MMPs) have been extensively studied in the context of vascular injury and in ventricular remodelling following ischaemia/reperfusion injury. However, more recently, MMPs have been demonstrated to have intracellular targets—cytoskeletal [1, 34], contractile [31, 35] and potentially also in terms of cell survival and cell death pathways [10, 20]. Robert Bell and Derek Yellon presented data demonstrating that not only does MMP inhibition at reperfusion attenuate infarction in both in vitro and in vivo preparations, but the protection observed is additional to that seen following targeted deletion of the cyclophilin-D component of the mPTP. The pharmacological agent used, ilomastat, had no direct inhibitory effect on mPTP opening in response to ROS exposure, further suggesting a conditioning independent mechanism of protection. Interestingly, MMP inhibition with ilomastat did result in increased phosphorylation of Akt, ERK and serine 9 of GSK-3β. Whether this is mechanistic, is currently unclear, and the discussion centred on potential mechanisms which could be further investigated in due course.

Mitochondrial connexins

Connexin (Cx) 43 has long been associated with the function of gap junctions and is also found to be associated with mitochondria as a consequence of preconditioning signalling [6, 9, 26]. Rainer Schulz presented the latest data from his laboratory regarding the role of Cx43 in preconditioning signalling. The C terminus of the Cx43 protein has been found to be the target for multiple post-translational modifications by kinase-mediated phosphorylation (PKA, Src, MAPK, PKC, CKI and Akt) and S-nitrosylation directing Cx43 within the cell and regulating open probability of Cx43 formed hemichannels, the latter being present also in mitochondria [28]. Recruitment of Cx43 appears to lead to mK_{ATP} opening [21, 27] and subsequent recruitment of preconditioning signalling through modification of mitochondrial respiration [7] and ROS generation [13]. While Cx43 appears not to be involved in postconditioning [16], there are data to suggest that inhibition of Cx43 leads to a lower threshold to opening of the mPTP in response to calcium [4].

Preconditioning mitochondria: implications for cardioprotection

Marisol Ruiz-Meana presented data revealing that it is possible to directly condition the isolated mitochondria, preserving complex 1 and 2 functions, mimicking the mitochondrial respiratory preservation observed in intact heart following ischaemic preconditioning. The ability to preserve complex 1 respiration in mitochondria was independent of mK_{ATP} function and was still evident even in mitochondria isolated from cyclophilin-D knock out hearts. Interestingly, the data demonstrated that differing mitochondrial populations had variable capacity to be preconditioned, which appeared to correlate with the presence or absence of Cx43. Cx43 is largely found in subsarcolemmal mitochondria which can be conditioned [9], versus the relatively deplete interfibrillar mitochondria which demonstrated little capacity to preserve complex 1 and 2 respiration following conditioning. Moreover, Cx43 knockouts show no preservation of complex 2 respiration following ischaemic preconditioning.

The mechanism of mitochondrial conditioning was unclear; during the discussion, oxidative modification of a cysteine residue of Cx43 during preconditioning was speculated along with the suggestion that there may still be kinase signalling activity within isolated mitochondria that explains the alterations of oxidative metabolism within preconditioned mitochondria.

Beta blockade and cardioprotection

The role of beta blockers and cardioprotection had been investigated in the past, and the data at that time felt unconvincing, but new work from Borja Ibanez's group suggests a new direction in studying a potentially cardioprotective role for beta blockade in ischaemia/reperfusion injury. In recent work, intravenous metoprolol was found to attenuate ischaemia/reperfusion injury concomitant with phosphorylation of Akt and attenuation of caspase-3 only when administered before reperfusion [19]. The postulated explanation for this protective effect was through activation of a recently characterised β_3 $G_{i/o}$-coupled receptor, which has been shown to increase the bioavailability of nitric oxide through endothelial nitric oxide synthase. Data were presented revealing that β_3-agonists result in significant attenuation of ischaemia/reperfusion injury [improved salvage index by cardiac magnetic resonance (CMR) imaging], improved left ventricular ejection fraction through different imaging modalities, and similar

improvements in viability isolated cardiac myocytes. In mouse, knockouts of β_3 show no such protection.

During the discussion, it was noted that similar results have also been seen using nebivolol [3], which recruits nitric oxide synthesis through β_3-receptor activation [14]. Curiously both the data presented and the nebivolol study appear in contrast to earlier data using various beta blockers (summarised by Hearse et al. [12]), and the reason for this discrepancy remains unclear. Two independent ongoing clinical trials (METOCARD-CNIC-clinical trial number NCT01311700 and EARLY-BAMI) are testing the revisited hypothesis that pre-reperfusion intravenous metoprolol administration (compared with post-reperfusion) will reduce infarct size in STEMI patients undergoing primary angioplasty. Other questions arose regarding the density of β_3-receptors in human myocardium, which at the present time is unknown, but work continues to further characterise this potential mechanism of protection and the potential clinical benefits in man.

Translating cardioprotection for clinical benefit

Insulin as an agent to protect against ischaemia–reperfusion injury

Lionel Opie presented an overview of metabolic therapy for acute myocardial infarction; following acute myocardial injury, adrenergic overdrive switches the substrate for myocardial metabolism from glucose to free fatty acids, which is deleterious in long-term myocardial viability. Sodi-Pallares et al. [33] were the first to describe the benefits of glucose/insulin/potassium (GIK) therapy in the context of acute myocardial infarction in 1962, and this has been an area of interest revisited many times over the following decades. One key aspect of insulin therapy, however, is the need for it to be administered early. In the recently reported Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency Care (IMMEDIATE) Trial [32], GIK was administered prior to admission to hospital in the ambulance and continued for 12 h thereafter and, therefore, present during early reperfusion. While the trial failed to meet its primary endpoint of progression to myocardial infarction, there was a significant reduction in the secondary endpoint, the composite of prehospital or in-hospital cardiac arrest or in-hospital mortality [32]. It is interesting to note that combinations of glucose, glucose/insulin and glucose/insulin/potassium have each been shown to have protective effect on all species studied, including man, as currently reviewed in detail [11].

The key point during the discussion of this presentation was the need for very early administration required for insulin's efficacy, both in terms of correcting the acute metabolic reaction to myocardial infarction, and in terms of recruitment of protective signalling cascades and modulation of mPTP opening.

Postconditioning: where do we go from here?

Ten years since presenting the original data showing the experimental evidence of ischaemic postconditioning, Jacob Vinten-Johansen reviewed the data showing how this phenomenon has progressed from the bench to the bedside, with evidence that not only does postconditioning result in attenuation of cell death by reducing necrosis and apoptosis through RISK pathways and inhibition of mPTP opening, but also ameliorates neutrophil adherence and coronary vascular endothelial dysfunction. Recent data have also inferred the presence of a delayed postconditioning phenomenon [5, 29], and further exploration and characterisation are required for this phenomenon which may represent attenuation of late onset inflammation and neutrophil infiltration. In terms of future targets for cardioprotection, the interaction between endothelium, neutrophils and platelets was discussed in terms of attenuating microvascular obstruction and post-infarction inflammation. The development of catheter-based devices for instigating ischaemic postconditioning and targeted myocardial hypothermia was suggested as a novel technology to induce cardioprotection through controlled reperfusion in patients undergoing primary percutaneous intervention.

The discussion centred on the proposed model of two waves of reperfusion injury, with the late phase being mediated by inflammatory and gene-transcription-mediated cell damage, which could be amenable to a late postconditioning protocol. It was suggested that work be translated into a larger animal model which more closely recapitulates the time course of reperfusion injury pathology and catheter-based reperfusion, as small rodents were felt to exhibit very rapid progression of injury and, therefore, large animal models are more likely to be reflective of the injury observed following acute myocardial infarction in man.

DPPIV inhibitors and GLP-1 analogues: novel cardioprotective and anti-diabetic agents

Richard Shannon presented data regarding DPPIV inhibitors and GLP-1 analogues, which independently of their anti-diabetic properties appear capable of inducing cardioprotection via G-protein-coupled receptors that also demonstrate G_s/G_i switching through β-arrestin. GLP-1 appears negatively inotropic, independent of glucose uptake. Interestingly, two peptide analogues, 7-36 and

9-36, demonstrate differing properties which were either p38 MAPK dependent/NO independent or p38 MAPK independent/NO dependent, respectively. Both markedly attenuated free fatty acid utilisation by mitochondria without alteration of state-3 respiration and also demonstrated some evidence of mitochondrial respiratory chain uncoupling. Interestingly, DPPIV inhibition was not cardioprotective at levels of normoglycaemia, unlike the protective properties of GLP-1 to improve post-ischaemic contractile recovery.

These classes of agents would appear to have considerable potential in the management of both diabetes and in cardioprotection. Whether DPPIV inhibitors would be cardioprotective in conditions of hyperglycaemia is currently unknown.

Ischaemic and pharmacological postconditioning: clinical application

Reinier Beeuwkes started this session describing a novel substance, CMX-2043, a lipoic acid, that appears to have cardioprotective properties through recruitment of Akt. CMX-2043 has recently been through a phase 2a trial and shown to be safe in the context of elective percutaneous intervention, and demonstrated some promise in attenuating troponin release when administered 15–120 min prior to the intervention.

David Garcia-Dorado provided an update on the soon to be reported Myocardial Protection With Adenosine During Primary Percutaneous Coronary Intervention in patients With STEMI (PROMISE) trial (clinical trial number NCT00781404). With the aim to evaluate the safety and efficacy of a brief intracoronary infusion of adenosine administered at the time of reperfusion and to assess the drug's efficacy to limit infarct size and left ventricular remodelling in patients undergoing primary percutaneous intervention for ST elevation myocardial infarction, this multicentre, prospective, randomised, placebo-controlled, double-blind study recruited 200 patients above 18 years of age with ST elevation, without prior myocardial infarction receiving primary PTCA within 6 h after the onset of symptoms. Infarct size and risk zone will be assessed by CMR, and change of left ventricular ejection fraction and end-diastolic diameter assessed at 6 months. Only very preliminary data were available by the time of the workshop, but the full data set is expected to be available by September 2012.

The Cyclosporine and Prognosis in Acute Myocardial Infarction (MI) Patients (CIRCUS) trial update was presented by Michel Ovize (trial number NCT01502774). This is a multicentre, randomised and double-blind trial designed to compare cyclosporine versus placebo administered as an intravenous bolus prior to restoration of blood flow by percutaneous coronary intervention. The study investigators are currently recruiting at 1 per month, with over 400 patients already recruited, and enrolment is expected to continue for another year. The primary endpoint is a combined incidence of total mortality, hospitalisation for heart failure and LV remodelling (defined as an increase of LV end-diastolic volume >15 % by transthoracic echocardiography), with secondary endpoints including all-cause mortality, cardiovascular death, heart failure, unstable angina or stroke.

Hans Erik Bøtker presented an update on the Effect of Remote Preconditioning in Primary Percutaneous Intervention of Acute ST Elevation Myocardial Infarction (CONDI) trial (NCT00435266). This randomised, single-blind trial used pressure cuff inflations to induce remote conditioning (4 cycles of 5 min ischaemia, 5 min reperfusion) in the transit ambulance prior to the arrival at the primary intervention centre following diagnosis of ST elevation myocardial infarction. The investigators recruited 333 patients grouped into treatment and placebo groups. Median salvage index was 0.75 in the remote conditioning group versus 0.55 in the control group. Moreover, there was an improvement in major adverse coronary and cerebral events (MACCE) in the RIPC group, presenting as an initial benefit and persisting over the subsequent 2 years. Therefore, it was found that remote ischaemic conditioning before hospital admission increased myocardial salvage and had a favourable safety profile.

The Effect of Remote Ischaemic Preconditioning on Clinical Outcomes in CABG Surgery (ERICCA) trial was presented by Derek Hausenloy. This randomised, single-blind, multicentre clinical trial compared four cycles of 5 min ischaemia, 5 min reperfusion of the upper limb induced by pressure cuff inflation prior to cardiac surgery (coronary artery bypass ± valve replacement), with the primary outcome of combined cardiovascular death, myocardial infarction, revascularisation and stroke. Secondary endpoints include peri-operative myocardial injury, LV ejection fraction, acute kidney injury, all-cause death, length of intensive care unit stay and quality of life. So far, the investigators had recruited 382 from a targeted 1,600 patients over 18 sites, with the aim to complete recruitment by October 2013, with results anticipated by December of that year.

Analysis of existing clinical evidence for conditioning

Gerd Heusch undertook to look at the translational clinical data centring upon cardioprotection and conditioning. Interestingly, of the clinical studies identified, almost all showed an attenuation in cardiac enzyme release in the context of ischaemic preconditioning in coronary artery bypass grafting, of ischaemic postconditioning in percutaneous intervention for ST elevation MI and of remote

ischaemic conditioning for both coronary artery bypass grafting and percutaneous coronary intervention [15]. The one common element identified underlying negative trial data with remote conditioning in surgery was the concomitant use of propofol. Other typical confounders that may influence clinical outcome are co-morbidities such as age [8] and diabetes [36], but an overlooked factor is the coronary vasculature both in terms of function and pathology [18]: slow reperfusion is itself cardioprotective [22] and microembolisation (that occurs following dispersal of the occlusive coronary thrombus) can attenuate preconditioning's protection [17]. Experimental microembolisation with inert microspheres of 40 μm diameter can increase infarct size by up to 15 %, predominantly through deposition of microparticles in the border zone surrounding the infarcted myocardium, although interestingly ischaemic postconditioning tends to ameliorate microsphere border zone deposition, suggesting preservation of microvascular function.

While ischaemic conditioning appears to have robust supporting clinical data, pharmacological conditioning, except where the end-effector, the mPTP, has been targeted (cyclosporine), has been rather disappointing. It was speculated that the transient ischaemic stimulus is more likely to recruit the full conditioning response, rather than a highly selective pharmacological trigger which may recruit only limited signalling.

Over all, it was felt that many of the early phase 2 and 3 trials of ischaemic conditioning had shown great promise, and perhaps the lack of general clinical adoption of conditioning was rather more dependent on the lack of significant clinical outcome trials, a void that hopefully will be filled once the current on-going clinical trials (as mentioned above) are reported.

Conclusions

The workshop has offered a preview of a number of original approaches to tackle cardioprotection in the future, from novel concepts in signal transmission both in the context of remote conditioning and platelet-derived signalling, through mPTP-independent cardioprotective signalling, to mitochondrial recruitment as an end-effector in the experimental setting, both in terms of mPTP function and mitochondrial morphology. In clinical translation, there are a number of exciting trials currently undergoing recruitment and providing hard primary outcome data that will hopefully provide evidence that conditioning is an efficacious approach in man that will add to the data of completed trials that have been discussed. Overall, the participants were optimistic regarding the future of cardioprotection as a potential tool in the management of patients with myocardial ischaemia, to further augment the interventions and therapies already available to the clinician.

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