Heart, Lung and Circulation (2015) **24,** 306–318
1443-9506/04/$36.00
http://dx.doi.org/10.1016/j.hlc.2014.10.001

ORIGINAL ARTICLE

Cardio-protective Signalling by Glyceryl Trinitrate and Cariporide in a Model of Donor Heart Preservation

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Received 2 September 2014; received in revised form 30 September 2014; accepted 5 October 2014; online published-ahead-of-print 14 October 2014

Background Storage of donor hearts in cardioplegic solutions supplemented with agents that mimic the ischaemic preconditioning response enhanced their post-reperfusion function. The present study examines the minimisation of cell death and activation of pro-survival signalling directed towards maintenance of mitochondrial homeostasis in hearts arrested and stored in two such agents, glyceryl-trinitrate, a nitric oxide donor and cariporide, (a sodium-hydrogen exchange inhibitor).

Methods After baseline functional measurement, isolated working rat hearts were arrested and stored for 6 h at 4 °C in either Celsior®, Celsior® containing 0.1 mg/ml glyceryl-trinitrate, 10 μM cariporide or both agents. After reperfusion, function was remeasured. Hearts were then processed for immunoblotting or histology.

Results Necrotic and apoptotic markers present in the Celsior® group post-reperfusion were abolished by glyceryl-trinitrate, cariporide or both. Increased phosphorylation of ERK and Bcl2, after reperfusion in groups stored in glyceryl-trinitrate, cariporide or both along with increased phospho-STAT3 levels in the glyceryl-trinitrate/cariporide group correlated with functional recovery. Inhibition of STAT3 phosphorylation blocked recovery. No phospho-Akt increase was seen in any treatment.

Conclusions Activation of signalling pathways that favour mitophagy activation (ERK and Bcl2 phosphorylation) and maintenance of mitochondrial transition pore closure after reperfusion (STAT3 and ERK phosphorylation) were crucial for functional recovery of the donor heart.

Keywords Donor heart • Ischaemia reperfusion injury • Pharmacological conditioning • Mitochondria • Sodium hydrogen exchange inhibitor • Glyceryl trinitrate

Introduction

Minimisation of ischaemia-reperfusion injury (IRI) incurred during the process of heart transplantation is essential for minimising primary graft failure and improving short- and long-term outcomes [1,2]. With the success of the transplant procedure, older and sicker individuals are now being accepted as recipients for cardiac transplantation resulting in a shrinking pool of ''standard criteria'' donor hearts. This has necessitated increased consideration and acceptance of

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''extended criteria'' or ''marginal'' donor hearts for transplantation. Hearts from older donors, those exposed to extended periods (> 4 h) of cold ischaemia (storage), or those exposed to large escalating doses of catecholamines after brain death make up the bulk of the ''extended criteria'' group [2,3]. Most cardioplegic/storage solutions have been in widespread use since the early 1990's and provide adequate protection for ''standard criteria'' donor hearts subjected to cold ischaemia times of less than four hours [4,5]. The capacities of these formulations to protect the donor heart may be suboptimal for the ''extended criteria'' group, given that these hearts are more susceptible to IRI incurred as a consequence of donor brain death and prolonged cold ischaemia [6].

The search for an over-arching protective strategy against cardiac IRI has been advanced by the elucidation of the process of ''cardiac conditioning''. Short non-lethal cycles of ischaemia and reperfusion or pharmacological agents mimicking these physiological strategies that are applied before (pre-conditioning), during (per-conditioning) or after (post-conditioning) an index period of ischaemia produce significant functional improvement during subsequent reperfusion [7]. Experimental studies indicate that such protective strategies initially employed against myocardial infarction are also effective in models of donor heart preservation and transplantation [8,9]. Previous work from our laboratory has shown that ischaemic preconditioning or the presence of pharmacological agents such as nitric oxide donors (diazenium diolates or glyceryl-trinitrate (GTN)), K_{ATP} channel openers or the sodium hydrogen exchange inhibitor cariporide at cardioplegia and during hypothermic storage significantly improved post-storage cardiac function in an isolated working rat heart model of donor heart preservation [10–12]. We have verified these studies in a porcine orthotopic transplant model incorporating six hours brain death where we have shown that donor hearts arrested and stored in Celsior® supplemented with GTN and cariporide for 14 h were able to be implanted into a recipient animal and successfully weaned from cardiopulmonary bypass [13].

Significant advances in codifying the mechanisms of cardiac conditioning have been achieved. The cardio-protective effect of activation of endogenous pro-survival signalling pathways (specifically Akt, ERK 1/2 and STAT3) by physiological and pharmacological pre-, per- and post-conditioning strategies has suggested a range of approaches to minimise IRI [14]. Consistent with this mechanism, we have shown that post-storage recovery of contractile function of hearts stored for six hours was associated with (i) Akt activation when arrested and stored in Celsior supplemented with the PARP inhibitor, INO1153; (ii) activation of Akt, ERK and STAT3 when hearts were arrested and stored in the presence of neuregulin [summarised in 2]; (iii) STAT3 and ERK activation when hearts were arrested and stored in the presence of the second generation sodium hydrogen exchange inhibitor, zoniporide [15]; and, (iv) activation of Akt, ERK and STAT3 when hearts were arrested and stored in the presence of EPO [16].

These findings prompted us to examine the extent to which the proximal pro-survival signalling pathways were activated and their potential downstream targets were effected in hearts arrested and stored in Celsior® supplemented with GTN and cariporide. Several other findings lend weight to the evaluation of pro-survival pathways in the protective effects of these agents. Firstly, p21 Ras, an upstream regulator of ERK and (potentially) Akt can be activated by nitric oxide through S-nitrosylation of the Cys^{118} of Ras [17]. Also, Ras and ERK could be activated by a transient intracellular acidosis [18], similar to that encountered in hearts exposed to cariporide immediately after reperfusion. Finally, an exogenous NO donor was shown to mimic eNOS-derived NO by activating the ERK pathway and STAT3 phosphorylation in a murine model of ischaemic preconditioning [19].

The present study employed an isolated working rat heart model of donor heart preservation with a six hour period of hypothermic storage. The following parameters were assessed in addition to functional recovery: 1) extent of necrosis; 2) extent of apoptosis (cleaved (activated) caspase 3; 3) phosphorylation status of ERK, Bcl2, Akt and STAT3 in the presence of GTN and cariporide compared to unsupplemented controls after storage and reperfusion and 4) the extent to which PD98059, an inhibitor of the MEK/ERK pathway or stattic, an inhibitor of STAT3 phosphorylation, modified the protective effect of GTN and cariporide.

Materials and Methods

Animals: Male Wistar rats (300-360 g) used in this study were obtained from the Animal Resource Centre (Canning Vale, Western Australia). Animals received humane care in compliance with the guidelines set down by the National Health and Medical Research Council (Australia). All procedures were approved by the Animal Ethics Committee of the Garvan Institute of Medical Research, Sydney, Australia (Animal Research Authority Ref Nos #03/24 and 06/25).

Chemicals and Pharmaceuticals: All chemicals unless otherwise stated were purchased from Sigma–Aldrich (St Louis, MO) [Analytical Reagent grade or equivalent]. Celsior® solution, was obtained from Genzyme (Naarden, The Netherlands). The sodium hydrogen exchange 1 inhibitor (HNEI-1), cariporide was a gift from Aventis Pharma (Frankfurt am Main, Germany). GTN was purchased from David Bull Laboratories (Victoria, Australia) as a 5 mg/ml solution. Ketamine was purchased from Parnell Laboratories (Alexandria, NSW, Australia), xylazine from Troy Laboratories, (Smithfield, NSW, Australia) and heparin from Pfizer Pty Ltd (North Ryde, NSW, Australia). All antibodies were sourced from Cell Signalling Technology, (Danvers, MA) with the exception of the phosho-Bcl2 antibody, obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Experimental Model: The isolated working rat heart model of donor heart preservation used in this study, has been previously described fully by us [15]. As illustrated in Figure 1, isolated rat hearts were stabilised for 10 mins by retrograde (Langendorff) perfusion with Krebs–Henseleit buffer at 37 °C, (composition (mM): NaCl 118; KCl 4.7;