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ORIGINAL PRE-CLINICAL SCIENCE

Improved heart function from older donors using pharmacologic conditioning strategies

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KEYWORDS:
pharmacologic conditioning;
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extended criteria hearts;
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myocardial preservation

BACKGROUND: Hearts from older donors are increasingly being referred for transplantation. However, these hearts are more susceptible to ischemia-reperfusion injury (IRI), reflected in higher rates of primary graft dysfunction. We assessed a strategy of pharmacologic conditioning, supplementing Celsior (Genzyme, Naarden, The Netherlands) preservation solution with glyceryl trinitrate (GTN; Hospira Australia Pty, Ltd, Mulgrave, VIC, Australia), erythropoietin (EPO; Eprex; Janssen-Cilag, North Ryde, NSW, Australia), and zoniporide (ZON; Pfizer, Inc., Groton, CT), to protect older hearts against IRI and improve graft function.

METHODS: Wistar rats, aged 3, 12, and 18 months old, were used to represent adolescent, 30-year-old, and 45-year-old human donors, respectively. Animals were subjected to brain death (BD) and hearts stored for 6 hours at 2° to 3°C in Celsior or Celsior supplemented with GTN+EPO+ZON. Cardiac function and lactate dehydrogenase before and after storage were assessed during ex vivo perfusion. Western blots and histopathology were also analyzed.

RESULTS: After BD, 18-month hearts demonstrated impaired aortic flow, coronary flow, and cardiac output compared with 3-month hearts ($p < 0.001$ to $p < 0.0001$). After storage in Celsior, the recovery of aortic flow, coronary flow, and cardiac output in 18-month BD hearts was further impaired ($p < 0.01$ vs 3-month hearts). Percentage functional recovery of 18-month BD hearts stored in Celsior supplemented with GTN+EPO+ZON was equivalent to that of 3-month hearts and significantly improved compared with 18-month hearts stored in Celsior alone ($p < 0.01$ to $p < 0.001$), with reduced lactate dehydrogenase release ($p < 0.01$) and myocardial edema ($p < 0.05$) and elevated phosphorylated extracellular signal–related kinase 1/2 ($p < 0.05$) and phosphorylated Akt ($p < 0.01$).

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CONCLUSIONS: Older hearts are more susceptible to IRI induced by BD and prolonged hypothermic storage. Supplemented Celsior activates cell survival signaling in older hearts, reduces IRI, and enhances donor heart preservation.
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The increasing demand for heart transplantation has led to the use of extended criteria organs, including those from older donors.[1] The mean age of cardiac donors has increased significantly during the last 2 decades, particularly in Australia and Europe, where the median donor age is now older than 40 years.[1–3] Registry data from the International Society for Heart and Lung Transplantation demonstrate that the risk of primary graft failure and early death after heart transplantation rises steadily above a donor age of 30 years.[4]

In addition, there is a powerful adverse interaction between donor age and ischemic time. Recipients of older-donor hearts subjected to prolonged ischemic times have the highest post-transplant mortality.[5,6] Pre-clinical studies have demonstrated that older hearts are more susceptible to ischemia-reperfusion injury (IRI) due to age-related dysregulation of mitochondrial oxidative phosphorylation and calcium handling, impaired antioxidant capacity, and changes in protein expression that result in myocardial injury, hypercontracture, and myocardial stunning.[7–11] Identifying strategies to overcome these aged-related defects in the cellular defence against IRI are essential if hearts from older donors are to become a safe and effective source of organs for transplantation.

One potential approach is pharmacologic conditioning, where cardioplegic and hypothermic preservation solutions are supplemented with pharmacologic agents that activate endogenous pro-survival–signaling pathways that offer protection against IRI.[12–15] These pathways include the reperfusion injury salvage kinase (RISK) and survivor activating factor enhancement (SAFE) pathways, which involve phosphorylation of key signaling proteins extracellular signal–related kinase (ERK) 1/2, Akt, signal transducer and activator of transcription (STAT) 3, and 5' adenosine monophosphate-activated protein kinase-α (AMPK-α).[13,14,16,17]

We previously demonstrated in small- and large-animal models of donor heart preservation that several pharmacologic agents enhance preservation of donor hearts when added to Celsior (Genzyme, Naarden, The Netherlands) preservation solution used in cardioplegia and hypothermic storage. These include glyceryl trinitrate (GTN; Hospira Australia Pty, Ltd, Mulgrave, VIC, Australia), a nitric oxide (NO) donor,[12,18–21] erythropoietin (EPO; Eprex; Janssen-Cilag, North Ryde, NSW, Australia), an activator of phosphoinositide 3-kinase–Akt and Janus kinase-STAT signaling cascade,[18,22] and zoniporide (ZON; provided by Pfizer, Inc., Groton, CT, under an independent external investigator agreement), a sodium-hydrogen exchange (NHE) inhibitor.[18,23–30] Moreover, the cardioprotective effects of these agents are additive, so that combining them as a triple supplement permits viable recovery of the donor heart after prolonged hypothermic storage.[18,22] These studies were performed on hearts obtained from young animals. Whether these cardioprotective benefits extend to hearts from older animals is unknown.

The aim of this study was to investigate whether these agents have similar protective effects in older hearts. Using an isolated working rat heart model, we initially assessed the effects of brain death (BD) and hypothermic storage on young and older hearts. We then assessed the efficacy of pharmacologic conditioning with EPO, GTN, and ZON to improve the preservation of cardiac function in older hearts.

Methods

This study was approved by the Animal Ethics Committee of the Garvan Institute of Medical Research, Sydney, New South Wales, Australia (Animal Research Authorities 12/25). Animals received humane care in compliance with the *Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes* (National Health and Medical Research Council, Australia) and the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, Bethesda, MD).

Male Wistar rats aged 3, 12, and 18 months (Charles River Laboratories, Wilmington, MA, and Animal Resource Centre, Canning Vale, Western Australia) were used to represent adolescent, 30-, and 45-year-old human cardiac donors,

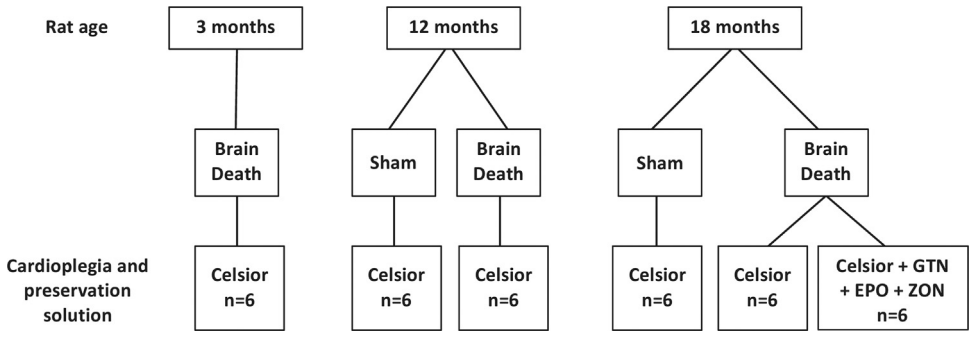

Figure 1 Study groups. EPO, erythropoietin; GTN, glyceryl trinitrate; ZON, zoniporide.