Use of Diffusion Tensor Imaging to Predict Myocardial Viability After Warm Global Ischemia: Possible Avenue for Use of Non-beating Donor Hearts

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Background: The assessment of myocardial viability after global warm ischemia (WI) but before reperfusion is challenging. We hypothesized that fractional anisotropy (FA), a magnetic resonance imaging (MRI) parameter of water diffusion that characterizes cellular integrity within tissues, provides a rapid and useful method for evaluating the viability of hearts after WI.

Methods: Dog hearts were exposed to 60 minutes of WI after exanguination, explanted and preserved in a cold, non-beating state for 6 hours, using continuous perfusion (CP) or static cold storage (CS). Toward the end of preservation, a global FA assessment, acquired using MRI, was compared with analyses obtained from myocardial biopsies that included adenosine triphosphate (ATP), endothelin-1 (ET-1) and caspase-3 levels, light microscopy and tetrazolium staining. Functional recovery was analyzed after restoration of blood flow on a non-working Langendorff preparation.

Results: FA measured at the end of CP showed strong correlations with all parameters of functional recovery (developed pressure, $R = 0.60$; dP/dt, $R = 0.96$; $-dP/dt$, $R = 0.96$). Although FA also correlated with tissue levels of ATP, ET-1 and caspase-3 ($R = 0.77$, -0.84, -0.64), recovery of myocardial function did not correlate with these markers or any other conventional analyses of myocardial injury (troponin I, changes on light microscopy or tetrazolium staining).

Conclusions: FA, an MRI-based parameter that indicates cellular integrity, was found to reflect better myocardial ATP stores, less induction of ET-1 and caspase-3 and improved functional recovery of hearts after global WI. As a clinically applicable tool capable of rapidly differentiating reversible from lethal injury, diffusion tensor imaging may prove useful in the eventual adoption of non-beating donor hearts for transplantation. J Heart Lung Transplant 2007;26:376–83. Copyright © 2007 by the International Society for Heart and Lung Transplantation.

The viability of cardiac tissue, as defined by the recovery of its function after a period of arrest, is adversely affected by prolonged periods of ischemia. Because of the clinical importance of this issue, there are numerous methods available for quantifying the effects of ischemia on the heart. Unfortunately, most of these methods (e.g., histology, myocardial enzyme release, etc.) require several hours after insult to discriminate myocardial injury and show limited sensitivity when used to evaluate the effects of ischemia without the benefit of reperfusion.

Cardiac transplantation is associated with an obligatory ex vivo transport period that can be exploited for appraising the viability of the marginal donor heart. The ability to define irreversible myocardial injury during the transport period would help greatly in the selection of hearts for transplantation. Although currently limited to heart-beating, brain-dead donors, the donor pool could be extended to include hearts from those dying from a cardiac death if the effects of global warm ischemia (WI) could be reliably discriminated before reperfusion. Although several studies have suggested that continuous perfusion (CP) techniques can be used

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Submitted July 17, 2006; revised November 10, 2006; accepted December 12, 2006.

Presented as a Philip Caves award finalist, International Society for Heart and Lung Transplantation, Madrid, Spain, April 2006.

Supported by grants from the Thoracic Surgery Foundation for Research and Education and the Office of Naval Research and an intramural grant from the University of Maryland.

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 1053-2498/07/$–see front matter. doi:10.1016/j.healun.2006.12.013

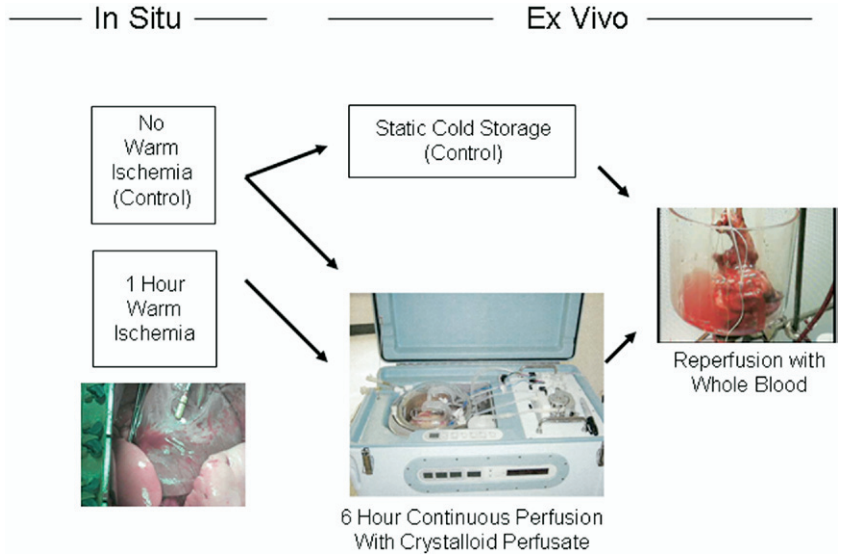

Figure 1. Experimental outline. Heart function was assessed in situ (baseline) and then dogs were exsanguinated by transecting the inferior vena cava. Hearts were either exposed to 1 hour of WI or immediately explanted and then preserved by continuous perfusion (CP) or static cold storage (CS). At the end of a 6-hour preservation interval, hearts were biopsied, weighed and assessed by diffusion tensor MRI. Hearts were reperfused with warm (37°C) whole blood on a non-working Langendorff preparation.

to "re-animate" the heart,[1-6] the risk of primary non-function after transplantation precludes the use of these organs clinically. The ideal way to predict the risk of non-function would be by global assessment of myocardial viability ex vivo during CP with non-invasive techniques that are readily available, rapid, and easy to perform.

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) technique that has the potential to meet all these criteria. DTI elucidates cellular viability by quantifying the directionality and magnitude of water diffusion within tissue fibers using a parameter called fractional anisotropy (FA). Brain DTI studies have shown reduced FA (i.e., poor directionality of water diffusion) in affected areas within minutes of stroke onset, often while conventional T1- and T2-weighted MRI images are normal,[7] and are accurate predictors of final infarct volume and neurologic prognosis.[7,8] Although technical difficulties associated with the imaging of moving tissue limit the application of DTI in the beating heart, donor hearts can be imaged ex vivo because the heart is motionless during the transport period. The hypothesis of this study was that FA measured during continuous ex vivo perfusion of hearts exposed to global WI would predict the viability and functional recovery of these hearts.

METHODS

Twenty-one mongrel dogs (20 to 30 kg) served as heart and blood donors for these studies. The protocol was approved by the Institutional Animal Care and Use Committee at the University of Maryland Medical Center. All animals received humane care and were housed in compliance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, Publication 85-23, revised 1985), and fed a normal diet.

Experimental Procedure

Surgery. Anesthesia was induced with intramuscular ketamine sodium followed by endotracheal intubation with isoflurane titrated for maintenance. Median sternotomy was used to expose the heart to obtain baseline biopsies and myocardial function using a intraventricular 9F Millar catheter placed through the apex. Heparin (300 IU/kg) was given and the dogs were exanguinated via the inferior vena cava with blood collected in citrate–phosphate–dextrose transfusion bags. After exanguination, hearts were exposed to global WI for 60 minutes, followed by 6-hour CP (WI + CP group, n = 9). Additional control hearts underwent cardioplegic arrest followed by immediate cardiectomy and then CP (CP group, n = 6) or CS (CS group, n = 6) for 6 hours. Before cardiectomy, all hearts were flushed with 1 liter of Celsior (Sangstat Corp., Fremont, CA) infused into the aortic root at a pressure of 65 mm Hg. After cardiectomy, the hearts were biopsied and weighed. The experimental design is summarized in Figure 1.

Myocardial Preservation

In the CS group, hearts were stored in iced (0° to 4°C) Celsior solution. Hearts from the CP group were perfused at a flow rate of 80 to 100 ml/min to maintain 15 mm Hg aortic pressure using a portable perfusion system (Organ Recovery Systems, Des Plaines, IL) that

includes a membrane oxygenator to maintain Pa_{O_2} at between 200 and 400 mm Hg. A catheter (13F Gundry RCSP, Medtronic, Inc.) was secured in the coronary sinus to provide access to the coronary effusate for sampling every 30 minutes during CP. A low-potassium perfusate at 4° to 6°C was used, consisting of $CaCl_2$ 0.5 mmol/liter, HEPES 10 mmol/liter, KPO_4 5 mEq/liter, mannitol 30 mmol/liter, glucose 10 mmol/liter, insulin 10 U, Na-gluconate 80 mmol/liter, magnesium 5 mmol/liter, ribose 5 mmol/liter, hydroxyethyl starch 50 g, glutathione 3 mmol/liter, adenine 5 mmol/liter, fructose-1,6-bisphosphate 10 mmol/liter, glutamic acid 26 mmol/liter, and chlorpromazine 0.2 mmol/liter, with pH titrated to 7.4 ± 0.05. Based largely on our own pre-clinical experience,[9] a brief mid-thermic interval was employed in which the temperature of the perfusate was raised from 4° to 25°C for 30 minutes and then cooled down to 4° to 6°C for the remainder of the preservation interval. Myocardial temperature was monitored with the Khuri pH monitoring system (Terumo Corp., Tokyo, Japan).

Langendorff Reperfusion

After preservation, electrolyte concentrations of autologous whole blood were corrected to physiologically normal values. Blood at 37°C was infused at 65 mm Hg into the aorta using a non-working Langendorff preparation. P_{O_2} (500 to 600 mm Hg) and P_{CO_2} (25 to 35 mm Hg) were maintained using a membrane oxygenator/heat exchanger (Optima, Cobe Cardiovascular) ventilated with a 95%–5% oxygenated CO_2 mixture. Myocardial function was assessed every 15 minutes during Langendorff reperfusion using an intra-ventricular 9F Millar catheter placed in a latex balloon inflated with saline to 0, 10, 20 and 40 ml. All pressure data were continuously recorded with a computer-based data acquisition system (PowerLab, ADInstruments, Inc., Colorado Springs, CO). The pressure vs time traces were analyzed via a previously described method of integrating trapezoidal areas under the curve[10] to determine the following LV functional parameters: developed pressure (DP); maximum rate of developed pressure ($+dP/dt$); and maximum negative rate of developed pressure ($-dP/dt$).

Diffusion Tensor Imaging

Diffusion tensor imaging was performed on the ex vivo hearts while CP was ongoing during the last 20 minutes of the preservation interval. Images were acquired on a 1.5-T MRI scanner (Philips Eclipse) equipped with echo-planar gradients using six co-linear directions of diffusion-weighting with an effective *b*-value of 1,000 s/mm^2. Other imaging parameters were: echo time/repetition time 96 ms/12,000 ms; matrix 80 × 80; field of view 20 cm^2; slice thickness 2 mm with no gap; and five averages. A total of 40 slices through the long axis of the heart were acquired. The fractional anisotropy values generated using standard equations[11] were obtained from four regions of interest (anterior, posterior, lateral and septal walls) in each of three separate slices. The average FA for these 12 regions was reported as the global FA value for that heart.

Graft Viability and Endothelial Function Assays

Myocardial biopsies were obtained from the anterior and posterior myocardial walls prior to cardiectomy (baseline) and at the end of the preservation interval (post-preservation) to determine ATP, endothelin-1 (ET-1) and caspase-3 levels. These assessments were performed with an ATP bioluminescent somatic cell assay kit (Sigma Chemical Co., St. Louis, MO), ET-1 enzyme immunometric assay kit (Assay Designs, Ann Arbor, MI) and caspase-3 chemiluminescence kit (Assay Designs). Histologic assessment of ischemic injury was done using hematoxylin–eosin (H&E) staining according to previously established criteria.[12] Tetrazolium staining was performed on myocardial slices using techniques previously described[13] and viability determined according to the presence of a deep red stain.

Statistical Analysis

The primary end-point of this trial was whether the global FA value obtained at the completion of the preservation period could predict the degree of functional myocardial recovery on the Langendorff analysis. Based on pilot data obtained in hearts without exposure to WI, we assumed a strong association between FA and markers of systolic recovery and a within-cell deviation in the data of <0.25. Therefore, power analysis indicated that six animals per group could provide 80% power to demonstrate this relationship at a 0.05 significance level. Nine animals in the WI group were chosen to allow for greater deviation in the data due to biologic variability in response to WI.

Variability in the data was quantified as the coefficient of variation = SD/mean. Statistical analysis was performed using statistical software (InStat 3.05 and Prism 4.0, GraphPad, Inc.). Student's *t*-test was used to evaluate differences in variables between groups. Correlations were examined using Fisher's exact test for non-parametric data and linear regression for parametric data, with $p < 0.05$ considered statistically significant.

RESULTS

Post-preservation Recovery

Although cardiac function was similar between groups at baseline (in situ), systolic and diastolic recovery on the Langendorff prep (ex vivo) varied widely, as summarized in Figure 2. The developed pressure (DP), rate

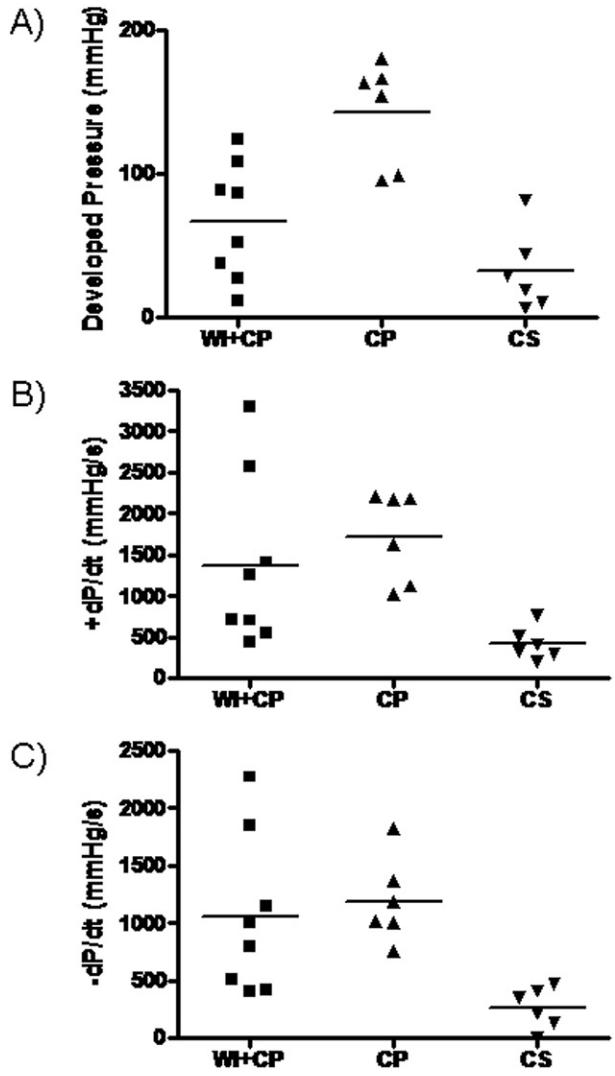

Figure 2. Systolic and diastolic function was determined by: developed pressure (DP) (A); rate of pressure generation (+d*P*/d*t*) (B); and maximum negative rate of developed pressure (−d*P*/d*t*) (C). Compared with hearts in the CS group, recovery was significantly better in hearts having undergone continuous perfusion, even in the WI + CP group. DP: 67.10 ± 40.59, 142.9 ± 36.40 and 31.94 ± 27.84 mm Hg; +d*P*/d*t*: 1,369 ± 1,041, 1,721 ± 547 and 420 ± 202 mm Hg; and −d*P*/d*t*: 1,051 ± 688, 1,191 ± 371 and 263 ± 177 mm Hg, for WI + CP, CP and CS, respectively ($p < 0.05$ for all).

of pressure generation (+d*P*/d*t*) and rate of relaxation (−d*P*/d*t*) were significantly worse ($p < 0.05$ for all) in hearts exposed to CS compared with WI + CP hearts. Despite superior recovery as a group, considerable inhomogeneity existed within the WI + CP group compared with other control groups, as reflected by an increased coefficient of variation for DP (0.60 vs 0.25), +d*P*/d*t* (0.70 vs 0.31) and −d*P*/d*t* (0.60 vs 0.31) for WI + CP vs CP alone ($p < 0.05$ for all).

WI hearts gained more weight after exposure to CP vs CS (16.6 ± 14.1 g vs 3.7 ± 5.2 g, $p < 0.05$). However, weight change did not correlate with any markers of myocardial viability or functional recovery.

Diffusion Tensor Imaging

FA maps were generated for WI hearts at the completion of CP (WI + CP, $n = 8$, one exam not completed due to technical reasons). Localized areas where water diffusion was not restricted, evident as discrete areas of signal hyperintensity (Figure 3A), were noted in those

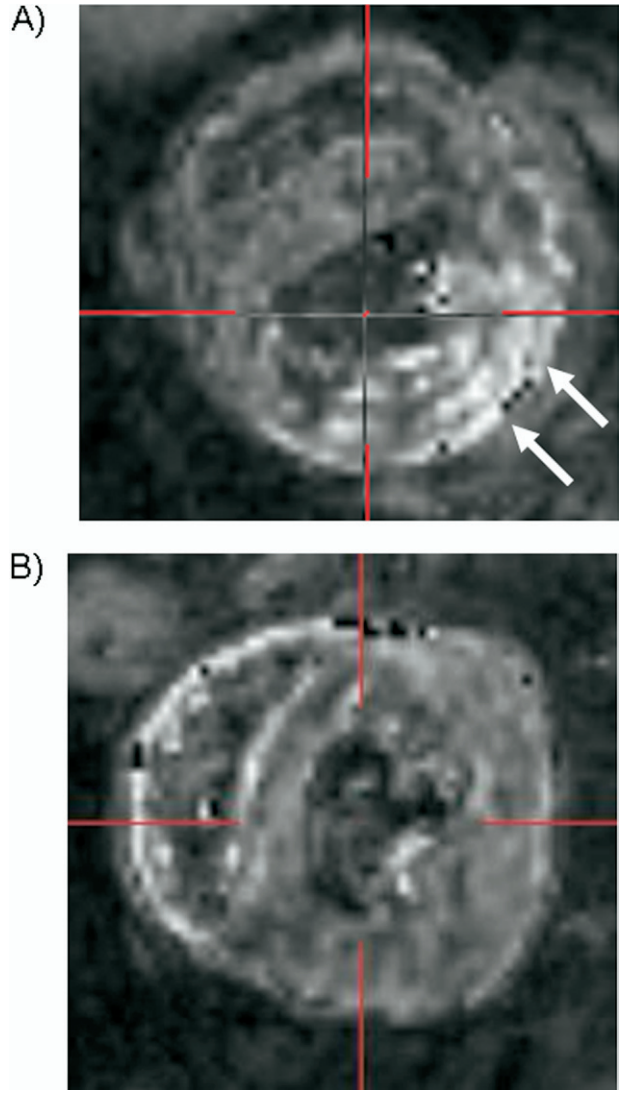

Figure 3. Diffusion-weighted maps of short-axis cross-sections of the donor heart were derived from DTI studies performed near the end of the preservation interval. After 60 minutes of WI, those hearts showing areas of hyperintensity suggestive of interstitial edema (A) (arrows) developed poor functional recovery after blood reperfusion on the Langendorff model. Maps showing more homogeneously restricted water motion (B) were seen in those hearts that developed better functional recovery, suggesting better tolerance to the initial ischemic insult and/or improved response to CP resuscitation.

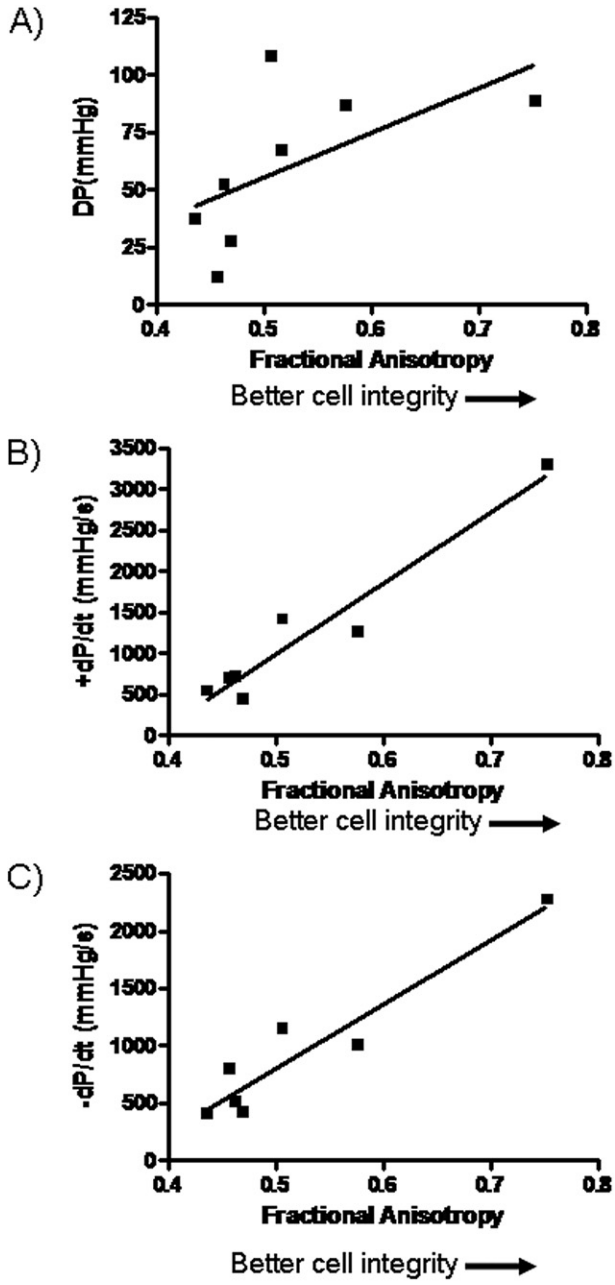

Figure 4. A global fractional anisotropy (FA) value was derived from the average readings obtained from 12 regions of interest for each heart and compared with systolic and diastolic function after Langendorff reperfusion. A strong association was noted between FA values and DP (A) ($R = 0.60$, $p = 0.079$), $+dP/dt$ (B) ($R = 0.96$, $p < 0.05$) and $-dP/dt$ (C) ($R = 0.96$, $p < 0.05$).

hearts developing poor systolic and diastolic recovery on the Langendorff preparation. By comparison, a homogeneous pattern of high FA (Figure 3B) was a predictor of improved functional recovery as evidenced by strong associations with all parameters of functional recovery: DP ($R = 0.60$, Figure 4A, $p = 0.079$); $+dP/dt$ ($R = 0.96$, Figure 4B, $p < 0.05$); and $-dP/dt$ ($R = 0.96$, Figure 4C, $p < 0.05$). In addition, FA correlated with the following traditional markers of myocardial viability: post-preservation ATP stores ($R = 0.77$, Figure 5A, $p < 0.05$); ET-1 levels ($R = -0.84$, Figure 5B, $p < 0.05$); and caspase-3 activity ($R = -0.64$, Figure 5C, $p = 0.08$).

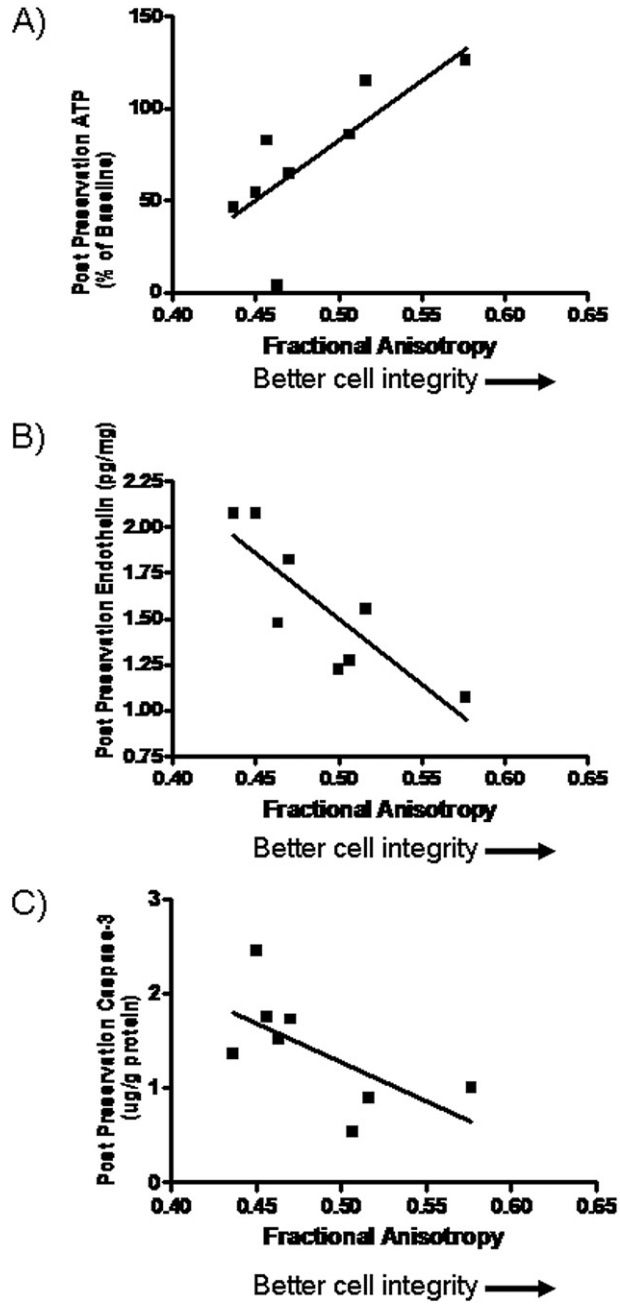

Figure 5. Fractional anisotropy (FA) values were correlated with other well-recognized markers of myocyte viability. Myocardial biopsies were obtained prior to cardiectomy (in situ) and at the end of the preservation interval (post-preservation) and assayed for ATP, endothelin-1 (ET-1) and caspase-3. Correlations were observed between FA and ATP (A) ($R = 0.77$, $p < 0.05$), ET-1 (B) ($R = -0.84$, $p < 0.05$) and caspase-3 (C) $R = -0.64$, $p = 0.08$).

Enzymatic and Biopsy-based Markers of Viability

In contrast to FA, standard techniques for determining myocardial viability did not show a relationship to functional recovery as measured during Langendorff reperfusion. Histologic sections, tetrazolium staining and troponin I from the WI + CP group showed no differences from control samples taken from hearts without WI in any of the sections analyzed. Troponin I was not detected in the coronary sinus during the CP interval in any of the groups. Even the levels of more sophisticated parameters of myocardial viability, ATP, caspase-3 activity and ET-1, did not show a significant correlation with any of the parameters of functional recovery.

DISCUSSION

Cardiac MRI is an increasingly common method currently used to clinically evaluate myocardial viability in patients with ischemic heart disease.[14,15] A limited donor pool for cardiac transplantation has led to the consideration of extended criteria donors and, potentially, organs from non–heart-beating donors (NHBDs).[16–19] However, the difficulties in defining the severity of ischemic injury and inability to assess graft function prior to transplant represents a significant barrier to the clinical use of these organs. We obtained a series of analyses including diffusion tensor MRI on the ex vivo asystolic heart as a model of the transport interval for a donor heart. Traditional biopsy-based markers of myocardial viability showed no relationship to the recovery of myocardial contraction and/or relaxation. Only FA was able to predict which heart was likely to recover after global WI in this clinically relevant large-animal model.

Even brief WI creates a severe energy deficit that can lead to an irreversible compromise in myocellular integrity. CP during the ex vivo transport interval led to the successful resuscitation of most hearts in the WI group, as evidenced by recovery of function back to baseline. However, a sub-set of the WI hearts did not recover, highlighting the clinical need for reliable methods that can effectively screen for hearts likely to develop primary graft dysfunction after transplantation. Water diffusion restricted to within intact myofibers is described as "anisotropic," reflected by DTI as an FA value close to 1. Therefore, a low FA value in the donor heart suggests processes such as extracellular edema and/or disrupted cell membranes are allowing less restricted water diffusion and, therefore, a higher risk of primary graft dysfunction after transplantation.

It is a well-described paradox that myocardial necrosis caused by WI requires full restoration of oxygenation and perfusion to manifest completely. As a result, conventional methods for defining necrosis, such as the release of myocardial enzymes or tetrazolium staining, have limited value for evaluating hearts exposed to ischemia but prior to restoring blood flow as required in this model. Classic histologic changes, such as contraction band necrosis, do not manifest for several hours after reperfusion,[20] and other studies using a continuous perfusion protocol found that routine tests for the assessment of creatine kinase MB could not reliably predict recovery after WI.[18,19] The assessment of histologic changes and troponin I release from WI + CP hearts in the present study corroborates the limitations of these methods.

Sophisticated biochemical assays of ATP stores,[1,6] ET-1[21] and caspase-3 provide more sensitive surrogates of injury. ET-1 a potent vasoconstrictor released from the myocardium after WI, is an established marker of endothelial injury[21] that has been associated with reduced survival after clinical heart transplantation.[22,23] ET-1 antagonists have been found to improve systolic function in animal models of cardiac transplantation.[23,24] Caspase-3 activity, a mediator of apoptosis, has been shown to correlate inversely with systolic recovery in rat hearts preserved using CP.[6] Inhibitors of apoptosis prolong the length of acceptable cold-storage time in animal models.[25] However, these assays are limited to a focal biopsy, making the data inherently prone to sampling bias and impractical for the restricted time available to complete an ex vivo organ evaluation. Although ATP stores and ET-1 levels have predicted systolic recovery after WI in other studies, only weak associations were seen in our model. DTI avoids the limitations of biopsy-based assays by providing a rapid and global assessment of the myocardium without increasing ischemic time using hardware (i.e., MRI) that is widely available. The cold, arrested, nonbeating donor heart presents an opportunity to investigate this technology for evaluating myocardial viability without the technical challenge of beating tissue. In addition to predicting functional recovery, FA showed significant correlations with ATP (Figure 5A), ET-1 (Figure 5B) and caspase-3 (Figure 5C), thereby strengthening the value of FA as a comprehensive means for evaluating the viability of ischemic myocardium. The findings of our model extend the established prognostic importance of FA values in brain tissue after stroke[8] into a new area in the ischemically damaged heart.

Study Limitations

The main limitation of this study is the use of the isolated heart model instead of orthotopic heart transplants to evaluate myocardial recovery. The non-working Langendorff model is less effective for analyzing diastolic function, a common cause of graft dysfunction after transplantation[26] and only provides data for the first 2 to 3 hours after restoration of blood flow. A major

advantage of the Langendorff model in this preliminary study is its ability to specifically address our study aim of showing a correlation of FA with myocardial recovery without the wide range of confounding effects present in a transplantation model. This method is well-established[22,27–29] and has a track record of predicting the clinical performance of a wide range of clinical protocols in cardiac surgery, including anti-ischemic interventions[28] and methods of cardiac preservation.[30,31] Furthermore, our model does not account for variables likely to be encountered in the clinical NHBD, including varying WI times, additional myocardial injury from donor hypoxia and hypotension prior to brain death, reperfusion with homologous (vs autologous) blood, and the lack of donor heparinization before cardiectomy.

Further studies are necessary to investigate the impact of these variables prior to use in the clinical setting. Although the heart rate and pre-load dependence of DP, $+dP/dt$ and $-dP/dt$ are well-known potential sources of experimental error, we found a consistent agreement between each of these functional parameters at varying pre-loads, thus providing confidence in the reliability of these data. Although suggested to be associated with interstitial edema in brain,[32] FA in our studies was not related to change in heart weight before and after the CP interval. Although WI + CP hearts gained more weight than CS hearts, weight change did not correlate with any of the other markers of functional recovery. The relationship of FA to extra- vs intracellular edema requires further clarification in this model.

In conclusion, the rapid evaluation of myocardial viability after ischemia has many potential applications beyond the evaluation of donor hearts before transplant. Unlike conventional means of assessing myocardial viability (e.g., release of myocardial enzymes, biopsy-based markers and light microscopy changes), only DTI was able to predict which hearts were likely to recover after sub-lethal WI. Although significant technical challenges remain regarding the expanded use of extended criteria donors, including NHBDs in the clinical setting, the results of this pre-clinical model warrant further investigation into the use of DTI as a means of assessing viability and predicting functional recovery during the ex vivo period.

The authors thank Organ Recovery Systems, Inc., for providing the modified KPS, RS-1, perfusion materials and technical expertise, and Sangsat for providing the Celsior preservation solution.

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