Brain-dead donor heart conservation with a preservation solution supplemented by a conditioned medium from mesenchymal stem cells improves graft contractility after transplantation

Sevil Korkmaz-Icöz1 | Kunsheng Li1 | Sivakkanan Loganathan1,2,3 | Qingwei Ding1 | Mihály Ruppert1,4 | Tamás Radovits4 | Paige Brlecic | Alex A. Sayour1,4 | Matthias Karck1 | Gábor Szabó1,3

Hearts are usually procured from brain-dead (BD) donors. However, brain death may induce hemodynamic instability, which may contribute to posttransplant graft dysfunction. We hypothesized that BD-donor heart preservation with a conditioned medium (CM) from mesenchymal stem cells (MSCs) would improve graft function after transplantation. Additionally, we explored the PI3K pathway’s potential role. Rat MSCs-derived CM was used for conservation purposes. Donor rats were either exposed to sham operation or brain death by inflation of a subdural balloon-catheter for 5.5 hours. Then, the hearts were explanted, stored in cardioplegic solution-supplemented with either a medium vehicle (BD and sham), CM (BD + CM), or LY294002, an inhibitor of PI3K (BD + CM + LY), and finally transplanted. Systolic performance and relaxation parameters were significantly reduced in BD-donors compared to sham. After transplantation, systolic and diastolic functions were significantly decreased, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)-positive cells and endonuclease G positive cells were increased in the BD-group compared to sham. Preservation of BD-donor hearts with CM resulted in a recovery of systolic graft function ($dP/dt_{\text{max}}$: BD + CM: 3148 ± 178 vs BD: 2192 ± 94 mm Hg/s at 110 µL, $P < .05$) and reduced apoptosis. LY294002 partially lowered graft protection afforded by CM in the BD group. Our data suggest that PI3K/Akt pathway is not the primary mechanism of action of CM in improving posttransplant cardiac contractility and preventing caspase-independent apoptosis.

**Abbreviations:** ANOVA, one-way analysis of variance; BD, brain death; CM, conditioned medium; $dP/dt_{\text{max}}$, maximum rate of rise of left-ventricular pressure; $dP/dt_{\text{min}}$, maximum rate of fall of left-ventricular pressure; EDV, end-diastolic pressure; ESPVR, the slope of the LV end-systolic pressure-volume relationship; FGF, fibroblast growth factor; IRI, ischemia/reperfusion injury; LV, left-ventricular; LY, LY294002; MI, myocardial infarction; MSCs, mesenchymal stem cells; PBS, phosphate-buffered saline; PI3K, phosphatidylinositol-3-kinase; PK, prokineticin; PRSW, preload recruitable stroke work; PTEN, phosphatase and tensin homologue; Tau, time constant of the LV pressure decay; TdT, terminal deoxynucleotidyl transferase; TIMP, tissue inhibitor of metalloproteinase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; VEGF, vascular endothelial growth factor.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. American Journal of Transplantation published by Wiley Periodicals LLC on behalf of The American Society of Transplantation and the American Society of Transplant Surgeons.
1 | INTRODUCTION

Heart transplantation is the definitive therapy for patients with end-stage heart failure. Currently, brain-dead (BD) donors constitute the major source of hearts for transplantation. However, brain death causes hemodynamic deterioration and neuroendocrine alterations, including a catecholamine surge, leading to cardiac dysfunction. The pathophysiology of brain death from increased intracranial pressure involves compensatory arterial hypertension to ensure adequate cerebral perfusion pressure, tachycardia, and intense peripheral vasoconstriction due to an early catecholamine storm. After this hypertensive phase, there is a loss of sympathetic tone and subsequent peripheral vasodilation is noted. Additionally, brain death activates inflammatory systems. Taken together, this may affect both the quantity and quality of hearts available for transplantation. Additionally, myocardial ischemia/reperfusion injury (IRI) at the time of transplantation leads to further impairment, contributing to adverse short- and long-term graft outcome in the recipients. Key underlying subcellular factors involved in IRI include the rapid restoration of physiological pH, the oscillation of intracellular calcium concentrations, cardiomyocytes ATP depletion, the generation of reactive oxygen species (ROS) and inflammatory cytokines, and inflammatory cell activation. This can eventually lead to cell death by apoptosis and to organ dysfunction. Taken together, BD-donor hearts may be associated with higher rates of posttransplant graft failure. Current specialized organ preservation solutions allow a safe ischemic time up to 4 hours regarding graft survival rates. However, there is still no effective therapy to alleviate the adverse effects of myocardial IRI.

Bone marrow-derived mesenchymal stem cells (MSCs), pluripotent adult stem cells, have drawn much attention as a new therapeutic agent to protect against myocardial IRI. Originally, direct differentiation of MSCs into cardiomyocytes, endothelial cells or vascular smooth muscle cells, to replace the damaged tissue, or cell-cell contact have been proposed as the main therapeutic mechanism. However, the number of differentiated cells generated appears to be too minimal to justify the many beneficial effects MSCs have on transplantation. Recent reports have indicated that paracrine factors secreted by these cells, including growth factors, chemokines, and cytokines play a major role in the cardioprotective effect rather than engraftment itself. Consistent with the paracrine hypothesis, the administration of MSCs secretion at the onset of reperfusion after ischemia, has shown evidence of protection against myocardial IRI in an ex vivo model. Potential paracrine mechanisms through which MSCs exert their therapeutic effect include the activation of endogenous tissue repair, stimulation of angiogenesis, the attenuation of cardiac remodeling, and the reduction of apoptosis. Accordingly, a novel cell-free but paracrine effect based therapeutic strategy could protect against myocardial IRI.

Therefore, we hypothesized that conditioned medium (CM) from bone marrow-derived MSCs added to a preservation solution improves in vivo left-ventricular (LV) graft dysfunction associated with brain death and heart transplantation in our well-established rat model. Activation of the phosphoinositide-3-kinase (PI3K) pathway has been shown to play an important role in the protective effect of CM and to protect cardiomyocytes from apoptosis during reperfusion. However, whether this activation mediates in vivo protective effects of CM is still unclear. Next, we tested the hypothesis that one or more of the active factors in CM may activate the PI3K pathway and we evaluated the molecular mechanisms behind this novel therapeutic strategy.

2 | MATERIALS AND METHODS

See the Online Appendix.

2.1 | Animals

Male Lewis rats were obtained from Janvier Labs with prior approval by the appropriate institutional review committees (G122/14).

2.2 | Experimental groups

2.2.1 | Before transplantation

Donor rats were randomly assigned into two groups: (1) sham (n = 15), subjected to sham operation and (2) BD (n = 25), exposed to brain death by inflation of a subdural balloon catheter. All animals were monitored for 5.5 hours.

2.2.2 | After transplantation

At the end of the 5.5 hours donor rat monitoring period, hearts were arrested and stored for 1 hour in either cold custodial supplemented with a medium vehicle ([1] sham [n = 8] and [2] BD [n = 10] groups), or custodial supplemented with CM ([3] sham + CM [n = 7] and [4] BD + CM [n = 8] groups), or custodial supplemented with CM and LY294002, a specific nonselective inhibitor of PI3K ([5] BD + CM + LY [n = 7] group). Then, the hearts were heterotopically transplanted.
2.3 | Preparation of CM

Bone marrow-derived MSCs were prepared from young rats (8-12 week-old) and CM was obtained at Passage 3, as described elsewhere. The protein concentration of the CM was quantified with Bradford protein assay to ensure that equal concentrations (0.5 mg/mL) of CM were used.

2.4 | Antibody array

For simultaneous detection of the relative expression of 90 target proteins in CM, RayBio® Biotin Label-based rat antibody array 1 (BioCat GmbH, Heidelberg, Germany) was used.

2.5 | Sham-operated and BD donors

2.5.1 | Model of brain death

The experimental model was described elsewhere. Sham-operated and BD donors were monitored for 5.5 hours.

2.5.2 | LV cardiac function

LV cardiac function was measured using a 2F-microtip pressure-volume (PV) conductance catheter (SPR-838; Millar Instruments, Houston, TX), as described elsewhere.

2.6 | Rat model of heterotopic heart transplantation

2.6.1 | Surgical technique of heart transplantation

Transplantations were performed in an isogenic Lewis to Lewis rat strain model, as described elsewhere. In rat cardiac allografts, reperfusion after short periods of cold ischemia (30 minutes, 1 hour, 1.5 hours) already causes a significant tissue damage and the degree of this graft injury correlates with increased ischemia time. Quantification of the effects of CM was necessary to avoid severe damage; therefore, the duration between explantation and reperfusion was standardized to 1 hour, as previously described. After completion of the anastomoses, the heart was reperfused with blood in situ for 1.5 hours. The rebeating time (time to restoration of heartbeat) after reperfusion was also recorded.

2.6.2 | Functional measurement in the graft

As previously reported, 1.5 hours after the onset of reperfusion, LV systolic pressure, LV end-diastolic pressure, maximal slope of systolic pressure increment (dP/dt_{max}), and diastolic pressure decrement (dP/dt_{min}) were determined at different LV volumes.

2.6.3 | Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling staining and immunohistochemistry in the graft

We performed terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay to detect DNA-strand breaks, as previously described and immunohistochemical staining for endonuclease G (1:50 dilution, Abcam, Cambridge, United Kingdom).

2.6.4 | Protein expression

Myocardial protein expression was assessed by western blot. The ratio of cleaved to total caspase-3, phosphorlated-Akt to Akt, and phosphorlated-p70S6K to p70S6K were calculated. Additionally, western blot analysis for phosphatase and tensin homologue (PTEN) was performed (1:1000 dilution, Cell Signaling Technology Europe BV).

2.7 | Statistical analysis

All data are expressed as mean ± SEM. Statistical analyses of data were performed using GraphPad Prism 7.02 software (GraphPad Software, Inc, La Jolla, CA). Before statistical tests were applied, the Shapiro-Wilk normality test was used to assess normal distribution. For data with normal distribution, a two-sample Student’s t test was used to analyze the difference between sham and BD groups. If the normality test failed, a nonparametric Mann-Whitney test was applied. One-way analysis of variance (ANOVA) and Tukey’s post hoc test were carried out to detect differences among the five experimental groups. If the data failed the normality test, the nonparametric Kruskal-Wallis test followed by Dunn’s post hoc test were used. A value of P < .05 was considered indicative of significance.

3 | RESULTS

3.1 | CM characterization by protein array

As we recently showed, CM was characterized via antibody array and revealed the presence of 28 proteins involved in apoptosis, inflammation, or oxidative stress.
3.2 | Functional characterization of BD donors

Table 1 shows the hemodynamic parameters 5.5 hours after brain death induction. Heart rate, systolic and diastolic blood pressures, and mean arterial pressure were significantly lower in BD donors compared to the sham group. Brain death was associated with a significantly decreased LV contractile function, including indices of load-dependent (stroke volume, ejection fraction, cardiac output, stroke work, and $dP/dt_{\text{max}}$, $P < .05$) and load-independent parameters (the slope $E_{\text{map}}$ of the end systolic PV relationship, preload recruitable stroke work (PRSW), slope of the $dP/dt_{\text{max}}$/end-diastolic volume relationship, and maximal elastance, $P < .05$). Additionally, ventricular relaxation was significantly impaired in the BD-group compared to the sham-operated rats, evidenced by decreased ($dP/dt_{\text{min}}$) and prolonged LV pressure decay (Tau-g), $P < .05$ (Table 1).

### Table 1  Hemodynamic parameters 5 hours after sham-operation or brain-dead induction

| Parameter                              | Sham donors     | Brain-dead donors | $P$ value |
|----------------------------------------|-----------------|-------------------|-----------|
| Heart rate (beats/min)                 | $374 \pm 11$    | $330 \pm 10^*$    | .0023     |
| Systolic arterial blood pressure (mm Hg)| $132 \pm 4$     | $61 \pm 2^*$      | <.0001    |
| Diastolic arterial blood pressure (mm Hg) | $106 \pm 4$    | $34 \pm 2^*$      | <.0001    |
| Mean arterial pressure (mm Hg)         | $115 \pm 4$     | $43 \pm 2^*$      | <.0001    |
| Ejection fraction (%)                  | $70 \pm 3$      | $36 \pm 2^*$      | <.0001    |
| Cardiac output (mL/min)                | $14 \pm 2$      | $8 \pm 1^*$       | .0077     |
| Stroke work (mm Hg × µL)               | $4695 \pm 493$  | $2487 \pm 381^*$  | <.0001    |
| $dP/dt_{\text{max}}$ (mm Hg/s)         | $8118 \pm 664$  | $3450 \pm 158^*$  | <.0001    |
| $dP/dt_{\text{min}}$ (mm Hg/s)         | $9736 \pm 726$  | $3235 \pm 176^*$  | <.0001    |
| Tau (Glantz) (ms)                      | $11.1 \pm 0.4$  | $13.3 \pm 0.6^*$  | .0028     |
| $E_{\text{max}}$ (ESPVR) (mm Hg/µL)   | $5.9 \pm 0.7$   | $4.1 \pm 0.4^*$   | .0156     |
| PRSW (mm Hg)                           | $103 \pm 11$    | $56 \pm 3^*$      | .0009     |
| Slope of $dP/dt_{\text{max}}$/EDV (mm Hg/s/µL) | $136 \pm 16$ | $52 \pm 7^*$      | <.0001    |
| Maximal elastance (mm Hg/µL)           | $7.0 \pm 0.8$   | $4.5 \pm 0.7^*$   | .0040     |

Note: Data are expressed as mean ± SEM. Abbreviations: $dP/dt_{\text{max}}$ indicates maximal slope of systolic pressure increment; $dP/dt_{\text{max}}$/EDV, end-diastolic volume; $dP/dt_{\text{max}}$, maximal slope of diastolic pressure decrement; $E_{\text{max}}$ (ESPVR), the slopes of the left-ventricular end-systolic pressure-volume relationship; PRSW, preload recruitable stroke work; Tau, time constant of the left ventricular pressure decay.

* $P < .05$ vs sham.

3.3 | Effect of CM on the transplanted heart

3.3.1 | Rebeating time

Rebeating time after reperfusion was significantly increased in the BD-donors compared to the sham-operated group. CM was associated with a significant shortening of graft rebeating time in the BD group (Figure 1).

3.3.2 | LV graft function

After transplantation, significantly decreased LV systolic pressure, developed pressure, $dP/dt_{\text{max}}$, and rate pressure product were observed in the BD-donor hearts when compared with the sham-operated group, indicating decreased systolic function (Figure 2A-D). CM resulted in better systolic functional and myocardial work recovery of grafts in both sham and BD-groups compared to their respective controls. Furthermore, after transplantation decreased $dP/dt_{\text{min}}$ was observed in the BD hearts compared to the sham group (Figure 2E). Even though active relaxation ($dP/dt_{\text{min}}$) was significantly improved in the sham + CM group compared to the sham group, CM had no effect on the BD group (Figure 2E).

3.3.3 | Apoptosis in the graft

After transplantation, DNA fragmentation, as reflected by an increased number of TUNEL-positive nuclei and increased endonuclease G-positive cells was observed in the BD group when compared with sham-operated rats (Figures 3 and 4). Treatment with CM significantly reduced the DNA strand breaks and endonuclease G-mediated apoptosis in the grafts of BD hearts. After transplantation, the densitometric analysis of bands for cleaved/total caspase-3

**FIGURE 1** Rebeating time (time to restoration of heartbeat) after transplantation. Values are expressed as mean ± SEM. *$P < .05$ vs sham; **$P < .05$ vs BD.
ratio did not show a significant difference among the experimental groups (Figure 5).

### 3.3.4 Protein expression in the graft

After transplantation, western blot analysis demonstrated that the expression of PTEN, total Akt, phosphorylated p70S6K, p70S6K, and phosphorylated-p70S6K/p70S6K ratio did not differ among the groups (Figure 6A,C,E-G). However, an increased phosphorylated-Akt expression in the BD was observed compared with the sham-operated rats (Figure 6B). Nevertheless, an already high level of phosphorylated-Akt/Akt ratio did not further increase after treatment with CM in the BD + CM group (Figure 6D). Moreover, the addition of LY294002 partially reversed beneficial effects of CM by decreasing phosphorylated-Akt protein expression and phosphorylated-Akt/total Akt ratio (Figure 6B,D).

### 3.3.5 Role of the PI3K pathway in the protective effect of CM against graft dysfunction associated with BD and heart transplantation

To investigate the role of the PI3K pathway we evaluated the BD + CM group combined with a PI3K inhibitor (LY294002, at a concentration of 15 µmol/L). The addition of LY294002 partially reversed beneficial effects of CM by reducing the improved systolic function (Figure 2A-C), by increasing the decreased DNA breaks and DNA fragmentation in tissue sections, detected by TUNEL assay (Figure 3), by decreasing phosphorylated-Akt protein expression and...
phosphorylated-Akt/total Akt ratio (Figure 6B,D), and by increasing decreased endonuclease G-mediated apoptosis (Figure 4) seen in the BD + CM rats. However, LY294002 had no effect on rebeat-ing time (Figure 1), on diastolic function, or on protein expression of cleaved caspase3- as a ratio of total caspase-3 (Figure 5), PTEN, Akt, phosphorylated-p70S6K, p70S6K, and phosphorylated-p70S6K/ p70S6K ratio (Figure 6A,C,E-G).

4 | DISCUSSION

In this study, we investigated whether bone marrow-derived MSCs-CM added to a preservation solution could have beneficial effects on graft function in a rat heart transplantation model, using BD donors. The novelty of this work lies in the fact that Custodiol-supplemented with CM improves posttransplant contractility in BD grafts and reduces caspase-independent apoptosis. This protection is not only through PI3K-dependent mechanisms, suggesting involvement of more than one cell signaling cascade.

In cardiac transplantation, currently, hearts are usually obtained from BD donors. However, hemodynamic instability and/or cardiac dysfunction is described in more than 25% of the potential BD donors, and they are excluded from transplantation. For these organs, the preservation solutions have a greater significance and hypothermic storage is the most common method employed. Various studies have suggested that CM administration improves cardiac function following myocardial infarction (MI). MSCs release a vast array of proteins including cytokines, chemokines, growth factors, metabolites, and bioactive lipids, which may be responsible for some of the observed cardioprotective effects. The characterization of CM via antibody-based protein array analyses showed that anti-apoptotic tissue inhibitor of metalloproteinase (TIMP)-1, growth hormone/receptor, prokineticin (PK)-1, vascular endothelial growth factor (VEGF), activin A, and fibroblast growth factor (FGF) binding protein may be involved in cardiac protection and functional improvement observed in the present study. It has been demonstrated that TIMP-1 sustained-release gel therapy provides improvement of cardiac function and inhibits apoptosis in a
FIGURE 4  Effect of conditioned medium (CM) on endonuclease G expression in donor heart grafts after transplantation. (A) Representative images of endonuclease G immunohistochemistry (in red, ×40 magnification, bar = 20 µm) followed by (B) quantification of endonuclease G-positive cells. Values are expressed as mean ± SEM. *$P < .05$ vs sham; *$P < .05$ vs BD; *$P < .05$ vs sham + CM; *$P < .05$ vs BD + CM [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 5  Effect of conditioned medium (CM) on caspase-3 protein expression in brain-dead (BD) donor heart grafts after transplantation and the role of the PI3K pathway. Cleaved caspase-3/total caspase-3 ratio protein band densities in the graft. Values are expressed as mean ± SEM
model of ischemic cardiomyopathy in rats. Urayama et al. have shown that prokineticin receptor-1 signaling promotes cardiomyocyte survival and decreases apoptotic cell death following hypoxia. Furthermore, it has been demonstrated that intramyocardial injection of VEGF-carrying plasmid resulted in cardiac performance restoration, infarct size reduction, and cardiomyocyte apoptosis inhibition in a rat model of MI. Additionally, activin A protein has been shown to protect cultured myocytes from hypoxia/reoxygenation-induced apoptosis, and its systemic overexpression has been shown to protect the heart from IRI in mice. Additionally, FGF-21 has been shown to protect H9c2 cells against IRI, mainly through the Akt-GSK-3β-caspase-3-dependent pathway, and to prevent oxidative stress, resulting in decreased apoptosis and remodeling following MI. It should also be mentioned that soluble tumor necrosis factor-related apoptosis inducing ligand (TRAIL) receptors 1 and 2 not only mediate pro-apoptotic signals but can also promote cell type-dependent pro-survival and proliferation signals. Recombinant soluble TRAIL induces the activation of intracellular signaling pathways, such as ERK/MAPK, Akt, and NF-kB, which are known to promote the survival/proliferation of endothelial and vascular smooth muscle cells. Further investigations are required to examine how other identified factors could be involved in the protective effect CM has on global IRI when hearts from BD donors are used.

**FIGURE 6** Effect of conditioned medium (CM) on PI3K/Akt signaling pathway proteins in donor heart grafts after transplantation and the role of the PI3K pathway. (A) Phosphatase and tensin homolog (PTEN) (B) phosphorylated-Akt, (C) Akt, (D) phosphorylated-Akt/total Akt ratio, (E) phosphorylated-p70S6K, (F) p70S6K, and (G) phosphorylated-p70S6K/p70S6K ratio protein band densities in the graft. Values are expressed as mean ± SEM. *P < .05 vs sham; #P < .05 vs BD; $P < .05 vs sham + CM; ψP < .05 vs BD + CM
4.1 | Mechanisms underlying the cardioprotective effects of CM after transplantation when using hearts from BD donors

In vitro studies of Angoulvant et al suggested that the protection afforded by CM may result from the activation of the PI3K pathway in neonatal rat cardiomyocytes. To assess the role of this pathway, first, we evaluated the protein expression of Akt and its upstream and downstream effectors. Our western blot analysis showed that CM had no effect on expression of tested proteins. Apoptosis is one of the key factors involved in the pathogenesis of IRI and brain death, leading to tissue damage. Caspases are crucial mediators of apoptosis, and among them caspase-3 activation (as reflected by the presence of its cleaved form) is a reliable indicator of apoptotic rate, leading to cleavage of a large number of protein substrates, and responsible for apoptosis DNA fragmentation. Our results demonstrated that CM decreased DNA-strand breaks and caspase-independent nuclear DNA fragmentation in BD-donor hearts. However, it has no effect on caspase-3 immunoreactivity. Next, we examined the effect of a PI3K inhibitor, LY294002 at functional levels, on apoptosis and protein expression of the PI3K/Akt pathway components. The activation of Akt is commonly measured as the ratio of phosphorylated-Akt to total Akt. Especially in hearts of BD donors, where the Akt is nearly maximally activated, the treatment with CM still has protective effects, predominantly by PI3/Akt-dependent mechanisms. Our data have shown that the preservation of BD-donor hearts with LY29002 added to the preservation solution supplemented with CM led to decreased Akt activity, increased DNA-breaks, and DNA fragmentation (detected by TUNEL-assy, which is sensitive to apoptosis), reduced systolic function, and increased caspase-independent apoptosis (evidenced by endonuclease G immunoreactivity). Our results indicate that the activation of the PI3K/Akt pathway is not the primary mechanism of action of CM in improving graft contractile function and preventing caspase-independent apoptosis. It should be noted that also in sham-operated animals CM improved LV graft contractility and even myocardial relaxation in vivo, suggesting that CM may exert direct effects on myocardial function.

4.2 | Study limitations

First, the rat model of cardiac transplantation was selected as a suitable model to evaluate global IRI. However, the left ventricle is beating in an unloaded condition, and therefore it does not eject, allowing a faster recovery after IRI. Second, right ventricular structure and function were not assessed. Third, the possible adverse effects of alloimmune reactions that can be triggered by heart transplantation were not examined, as the current experiments were performed using an isimmune model to investigate IRI independent from acute rejection. Fourth, it would have been of great interest to identify candidate proteins detected in CM displaying protection. However, we do not have direct evidence at this point. Fifth, the impact of longer cold ischemia times was not assessed.

4.3 | Conclusions

The data reported in this in vivo study provide experimental evidence that the preservation of BD-donor hearts with cardioplegic solution-enriched with CM (rather than an injection) improves cardiac graft contractility after transplantation. The signaling through PI3K/Akt pathway is not the sole mechanism participating in the cardioprotection conferred by CM. Further investigation is required to identify the mechanisms of CM-induced protection. From the clinical point of view, there is an extreme necessity for improved hypothermic organ preservation solutions that afford enhanced protection against IRI by minimizing its adverse effects on graft function, and that optimize the use of all available organs. Various factors present in CM may represent a balanced “cocktail” acting together and through more than one mechanism to promote protection. Therefore, further studies are required to examine the effects of each candidate factor detected in CM, which may have important clinical implications in the development of the optimal preservation solution.

ACKNOWLEDGMENTS

This study was supported by the Land Baden-Württemberg, Germany, by the Medical Faculty of the University of Heidelberg, Germany (to Dr S. Korkmaz-Icöz), and by the National Research, Development and Innovation Office of Hungary (NKFIA; NVKP-16-1-2016-0017). The expert technical assistance of K. Sonnenberg, L. Hoffmann, T. Mayer, and P. Kraft is gratefully acknowledged. We thank the Center for Model System and Comparative Pathology (Dr T. Poth, K. Rebholz) for their support.

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available.

ORCID

Sevil Korkmaz-Icöz https://orcid.org/0000-0003-3613-5807

REFERENCES

1. Szabo G. Physiologic changes after brain death. J Heart Lung Transplant. 2004;23(9 Suppl):S223-226.
2. Hearse DJ, Bolli R. Reperfusion induced injury: manifestations, mechanisms, and clinical relevance. Cardiovasc Res. 1992;26(2):101-108.
3. Anversa P, Cheng W, Liu Y, Leri A, Redaelli G, Kajstura J. Apoptosis and myocardial infarction. Basic Res Cardiol. 1998;93(Suppl 3):s8-s12.
4. Stehlik, J., Edwards, L. B., Kucheryavaya, A. Y., et al. The Registry of the International Society for Heart and Lung Transplantation: twenty-seventh official adult heart transplant report-2010. *J Heart Lung Transplant*. 2010;29(10):1089-1103.

5. Young, J. B., Naftel, D. C., Bourge, R. C., et al. Matching the heart donor and heart transplant recipient. Clues for successful expansion of the donor pool: a multivariable, multiinstitutional report. The Cardiac Transplant Research Database Group. *J Heart Lung Transplant*. 1994;13(3):353-365.

6. Price, M. J., Chou, C.-C., Frantzen, M., et al. Intravenous mesenchymal stem cell therapy early after reperfused acute myocardial infarction improves left ventricular function and alters electrophysiologic properties. *Int J Cardiol*. 2006;111(2):231-239.

7. Shake, J. G., Gruber, P. J., Baumgartner, W. A., et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. *Ann Thorac Surg*. 2002;73(6):1919-1926.

8. Caplan, A. I., Dennis, J. E. Mesenchymal stem cells as trophic mediators. *J Cell Biochem*. 2006;98(5):1076-1084.

9. Gnecchi, M., He, H., Liang, O. D., et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med*. 2005;11(4):367-368.

10. Kinnaird, T., Stabile, E., Burnett, M. S., et al. Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation*. 2004;109(12):1543-1549.

11. Angoulvant, D., Ianes, F., Ferrera, R., Matthews, P. G., Nataf, S., Ovize, M. Mesenchymal stem cell conditioned media attenuates in vitro and ex vivo myocardial reperfusion injury. *J Heart Lung Transplant*. 2011;30(1):95-102.

12. Hegeduš, P., Li, S., Korkmaz-Icöz, S., et al. Dimethylxoyalglycine treatment of brain-dead donor rats improves both donor and graft left ventricular function after heart transplantation. *J Heart Lung Transplant*. 2016;35(1):99-107.

13. Li, S., Korkmaz-Icöz, S., Radovits, T., et al. Donor preconditioning after the onset of brain death with dopamine derivative n-octanoyl dopamine improves early posttransplant graft function in the rat. *Am J Transplant*. 2017;17(7):1802-1812.

14. Loganathan, S., Korkmaz-Icöz, S., Radovits, T., et al. Effects of soluble guanylate cyclase activation on heart transplantation in a rat model. *J Heart Lung Transplant*. 2015;34(10):1346-1353.

15. Hung, S. C., Pochampally, R. R., Chen, S. C., Hsu, S. C., Procop, D. J. Angiogenic effects of human multipotent stromal cell conditioned medium activate the PI3K-Akt pathway in hypoxic endothelial cells to inhibit apoptosis, increase survival, and stimulate angiogenesis. *Stem Cells*. 2007;25(9):2363-2370.

16. Fujio, Y., Nguyen, T., Wencder, D., Kitsis, R. N., Walsh, K. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. *Circulation*. 2000;101(6):660-667.

17. Korkmaz-Icöz, S., Li, S., Hüttrner, R., et al. Hypothermic perfusion of donor heart with a preservation solution supplemented by mesenchymal stem cells. *J Heart Lung Transplant*. 2019;38(3):315-326.

18. Li, S., Loganathan, S., Korkmaz, S., et al. Transplantation of donor hearts after circulatory or brain death in a rat model. *J Surg Res*. 2014;195(1):315-324.

19. Li, S., Korkmaz, S., Loganathan, S., et al. Acute ethanol exposure increases the susceptibility of the donor hearts to ischemia/reperfusion injury after transplantation in rats. *PLoS ONE*. 2012;7(11):e49237.

20. Tanaka, M., Mokhtari, G. K., Terry, R. D., et al. Prolonged cold ischemia in rat cardiac allografts promotes ischemia-reperfusion injury and the development of graft coronary artery disease in a linear fashion. *J Heart Lung Transplant*. 2005;24(11):1906-1914.

21. Korkmaz-Icöz, S., Li, S., Loganathan, S., et al. Impairment of the Akt pathway in transplanted Type 1 diabetic hearts is associated with post-transplant graft injury. *Interact Cardiovasc Thorac Surg*. 2018;27(6):884-894.

22. Korkmaz-Icöz, S., Szczesny, B., Marcatti, M., et al. Olaparib protects cardiomyocytes against oxidative stress and improves graft contractility during the early phase after heart transplantation in rats. *Br J Pharmacol*. 2018;175(2):246-261.

23. Timmers, L., Lim, S. K., Hoefer, I. E., et al. Human mesenchymal stem cell-conditioned medium improves cardiac function following myocardial infarction. *Stem Cell Res*. 2011;6(3):206-214.

24. Uchinaka, A., Kawaguchi, N., Mori, S., et al. Tissue inhibitor of metalloproteiase-1 and -3 improves cardiac function in an ischemic cardiomyopathy model rat. *Tissue Eng Part A*. 2014;20(21-22):3073-3084.

25. Urayama, K., Guilini, C., Messaddeq, N., et al. The prokineticin receptor-1 (GPR73) promotes cardiomyocyte survival and angiogenesis. *FASEB J*. 2007;21(11):2980-2993.

26. Ruxing, Y., Dezhai, Y., Hai, W., Kai, H., Xianghong, W., Yuming, C. Intramyocardial injection of vascular endothelial growth factor gene improves cardiac performance and inhibits cardiomyocyte apoptosis. *Eur J Heart Fail*. 2007;9(4):343-351.

27. Oshima, Y., Ouchi, N., Shimano, M., et al. Activin A and follistatin-like 3 determine the susceptibility of heart to ischemic injury. *Circulation*. 2009;120(16):1606-1615.

28. Cong, W.-T., Ling, J., Tian, H.-S., et al. Proteomic study on the protective mechanism of fibroblast growth factor 21 to ischemia-reperfusion injury. *Can J Physiol Pharmacol*. 2013;91(11):973-984.

29. Joki, Y., Ohashi, K., Yuasa, D., et al. FGF21 attenuates pathological myocardial remodeling following myocardial infarction through the adiponectin-dependent mechanism. *Biochem Biophys Res Commun*. 2015;459(1):124-130.

30. Chen, P. L., Easton, A. Apoptotic phenotype alters the capacity of tumor necrosis factor-related apoptosis-inducing ligand to induce human vascular endothelial activation. *J Vasc Res*. 2008;45(2):111-122.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Korkmaz-Icöz, S., Li, K., Loganathan, S., et al. Brain-dead donor heart conservation with a preservation solution supplemented by a conditioned medium from mesenchymal stem cells improves graft contractility after transplantation. *Am J Transplant*. 2020;20:2847-2856. [https://doi.org/10.1111/ajt.15843](https://doi.org/10.1111/ajt.15843)