UniversityHospital Heidelberg

Conservation of hearts from brain-dead donors with a preservation solution supplemented by a conditioned medium from mesenchymal stem cells, improves graft contractility after heart transplantation in rats

Sevil Korkmaz-Icöz[1], Kunsheng Li[1], Mihály Ruppert[1], Sivakkanan Loganathan[1], Tamás Radovits[2], Paige Brlecic[1], Matthias Karck[1], Gábor Szabó[1]

[1]Department of Cardiac Surgery, University of Heidelberg, 69120 Heidelberg, Germany
[2]Heart and Vascular Center, Semmelweis University, 1122 Budapest, Hungary

Financial Disclosures

None

Background

Introduction

Heart transplantation (HTx) is often the only treatment in end-stage heart failure. However, hemodynamic instability demonstrated in brain-dead (BD) donors may affect the posttransplant graft function after ischemia/reperfusion (IR). Previous studies have suggested that mesenchymal stem cell (MSCs) administration, or their conditioned medium (CM), protects the heart against IR injury. Additionally, in vitro data suggest a protection mediated by paracrine activation of the phosphatidylinositol 3 kinase (PI3K) pathway.

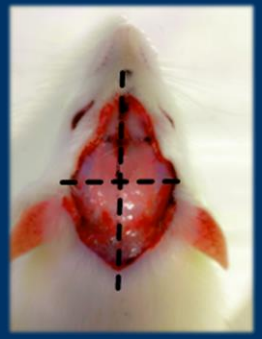
Hypothesis

We hypothesized that CM, when added to a preservation solution (Custodiol), will attenuate in vivo left-ventricular (LV) graft dysfunction after heart transplantation. Additionally, we explored the potential implication of the PI3K pathway.

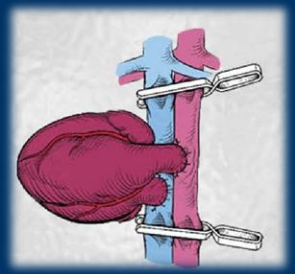
Methods

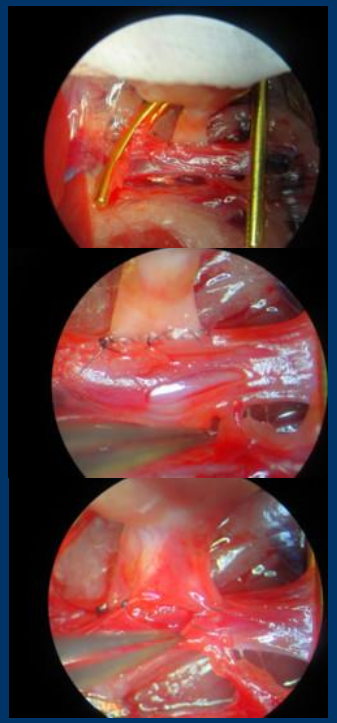
- In donor rats, BD was induced by a subdurally placed, and inflated balloon catheter.
- Controls were sham-operated.
- Sham-operated and BD donors were monitored for 5h.
- Hearts were arrested and stored for 1h in either cold:
  - Custodiol supplemented with medium vehicle
    - * BD group, n=10
    - * sham group, n=8
  - Custodiol supplemented with CM
    - * BD CM group, n=8
    - * sham CM group, n=7
  - Custodiol supplemented with CM and LY294002, a specific non selective inhibitor of PI3K
    - * BD CM+LY group, n=7
- Hearts were heterotopically transplanted.
- Posttransplant graft function was evaluated *in vivo* 1.5h after transplantation via a Millar catheter system.
- Histological and molecular biological measurements were also performed.

Donor rats

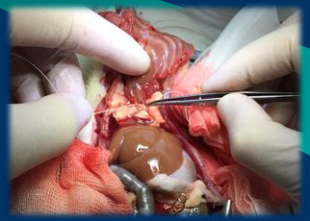


Subdurally placed balloon catheter



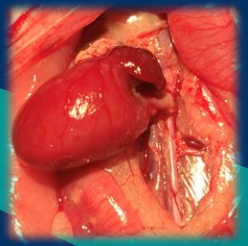


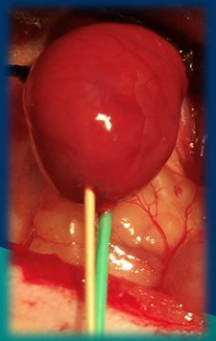
5h after sham-op or BD



- Measurement of LV cardiac function
- Explantation of hearts from donors
- Storage for 1h in cold preservation solution

HTx





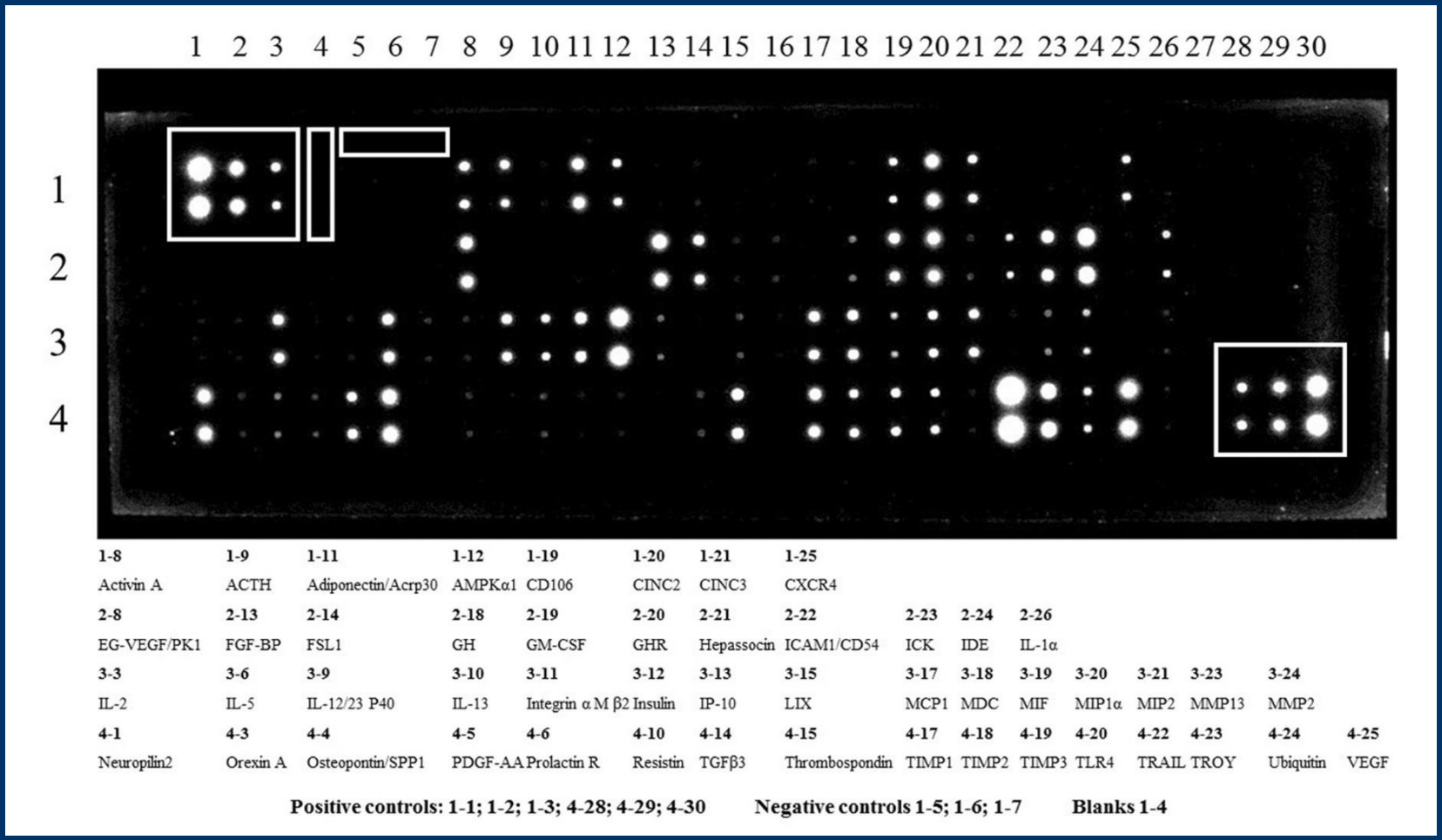
1.5h after transplantation

— LV graft function
— Explantation of heart for histological and molecular biological analyses

Characterization of MSCs-CM

Rat MSCs were isolated and cultured.

The CM at passage P3 was used for conservation purposes, and was characterized by an antibody array revealing the presence of 28 factors involved in apoptosis, inflammation, oxidative stress.



Factors detected in CM from bone marrow-derived MSCs

Phenomena	Specific properties	Factors
Apoptosis	Anti-apoptotic	TIMP1, Growth Hormone/-R, EG-VEGF (PK1), VEGF, Activin A
	Pro-apoptotic	TRAIL, Thrombospondin, TROY
Angiogenesis	Pro-angiogenesis	EG-VEGF (PK1), VEGF, MMP2, FGF-BP, Neuropilin2, PDGF-AA, MCP1
	Anti-angiogenesis	TIMP2, Thrombospondin, IP-10
Stress	Protection from oxidative stress	FGF-BP
Inflammation	Pro-inflammatory	CINC2/3, FSL1, MCP1, MDC, MIF, MIP1α, Osteopontin/SPP1
	Anti-inflammatory	MDC
	Immunmodulator	Osteopontin, GM-CSF, IP-10, LIX
Others	Cell proliferation	TIMP1
	Metabolism	Growth Hormone/-Receptor, IDE
	ECM modulation	TIMP1, TIMP3, MMP-13

Results

Hemodynamic changes and LV cardiac function after brain death

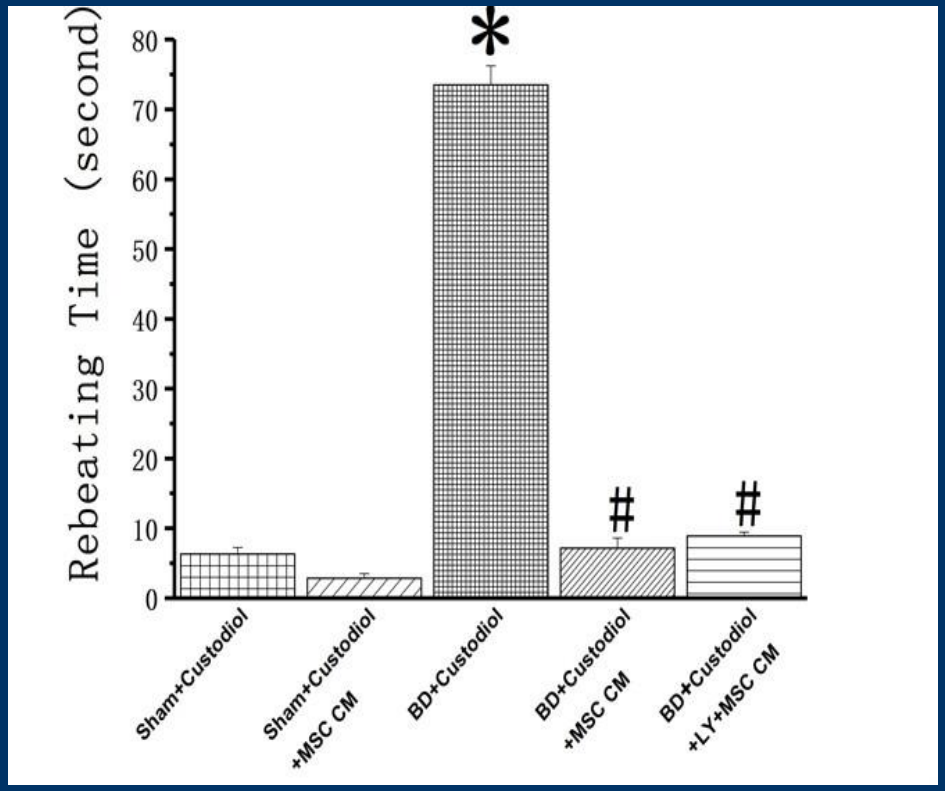
Parameters	Sham	Brain death	P value
Systolic blood pressure (mmHg)	132 ± 4	61 ± 2*	< 0.0001
Diastolic blood pressure (mmHg)	106 ± 4	34 ± 2*	< 0.0001
Mean arterial pressure (mmHg)	115 ± 4	43 ± 2*	< 0.0001
Heart rate (bpm)	374 ± 11	330 ± 10*	0.0023
Stroke Volume (µL)	37 ± 4	25 ± 4*	< 0.0001
Ejection Fraction (%)	70 ± 3	36 ± 2*	< 0.0001
Cardiac Output (mL/min)	14 ± 2	8 ± 1*	0.0077
Stroke Work (mmHg*µL)	4695 ± 493	2487 ± 381*	< 0.0001

Parameters	Sham	Brain death	P value
dP/dt_{max} (mmHg/s)	8118 ± 664	3450 ± 158*	< 0.0001
dP/dt_{min} (mmHg/s)	-9736 ± 726	-3235 ± 176*	< 0.0001
Tau (G) (ms)	11.1 ± 0.4	13.3 ± 0.6*	0.0028
Maximal Power (mWatts)	30 ± 3	12 ± 1*	< 0.0001
Preload adjusted maximal power (mWatts/µL^2)	98 ± 27	12 ± 2*	< 0.0001
ESPVR quadr E´ max (mmHg/µL)	5.9 ± 0.7	4.1 ± 0.4*	0.0156
PRSW (mmHg)	103 ± 11	56 ± 3*	0.0009
dP/dt_{max}-EDV (mmHg/s)/µL)	136 ± 16	52 ± 7*	< 0.0001
Max elastance (mmHg/µl)	7.0 ± 0.8	4.5 ± 0.7*	0.0040

Values are expressed as means ± SEM.
dP/dt_{max}=Maximum rate of rise of left ventricular pressure; dP/dt_{min}=maximum rate of fall of left ventricular pressure;Tau_g=Tau-Glantz method; ESPVR=End-systolic pressure-volume relationship; PRSW=Preload-recruitable stroke work; dP/dt_{max}-EDV= Maximum dP/dt - end-diastolic volume.

BD was associated with significantly decreased systolic performance and impaired cardiac relaxation.

Effect of CM on re-beating time after transplantation (time to restoration of heartbeat after transplantation) -– role of the PI3K pathway



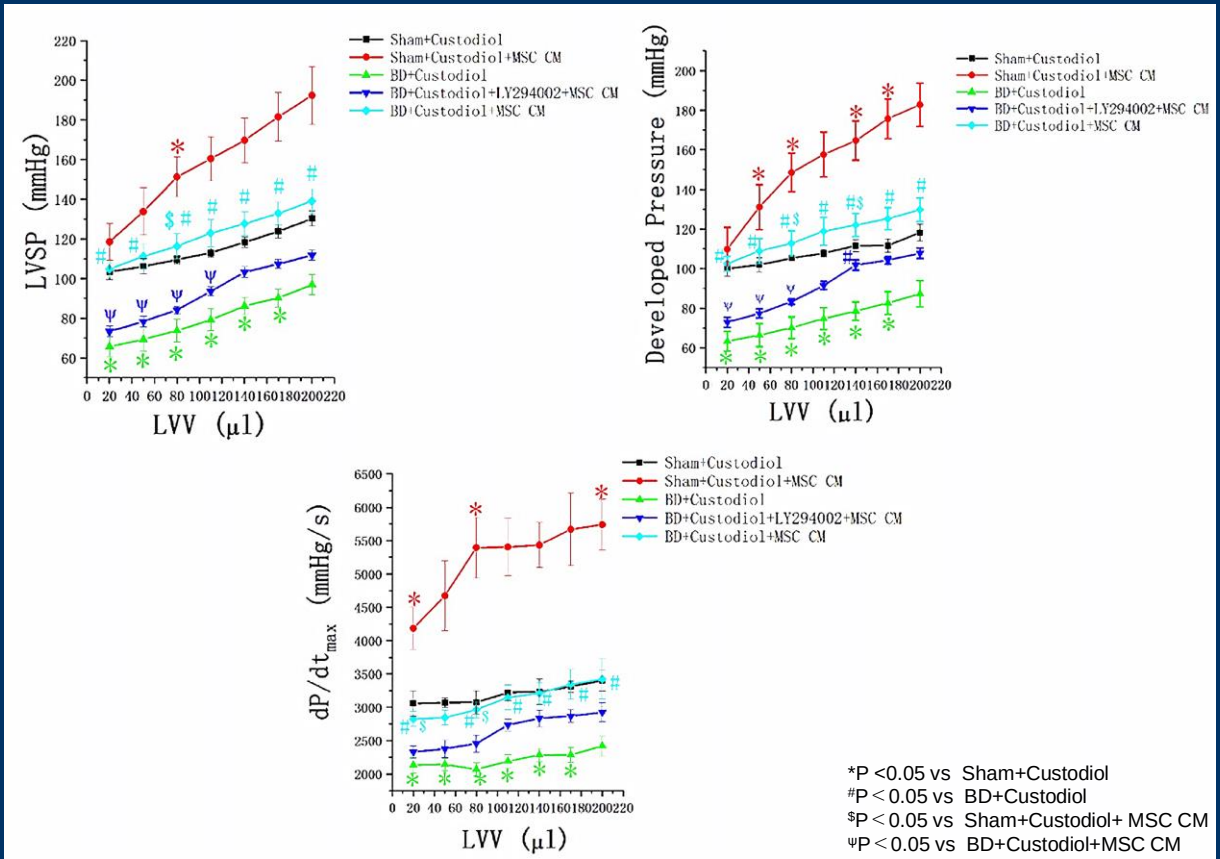
*P ＜0.05 vs Sham+Custodiol
#P＜0.05 vs BD+Custodiol
$P＜0.05 vs Sham+Custodiol-CM
ΨP＜0.05 vs BD+Custodiol-CM

Re-beating time after reperfusion was significantly increased in the BD group compared to the sham-operated group.

CM was associated with shortened graft re-beating time in the BD group.

Addition of a specific non-selective inhibitor of PI3K, LY294002, to the CM had no effect.

Effect of MSC CM on LV systolic function of the graft from BD donor hearts – role of the PI3K pathway

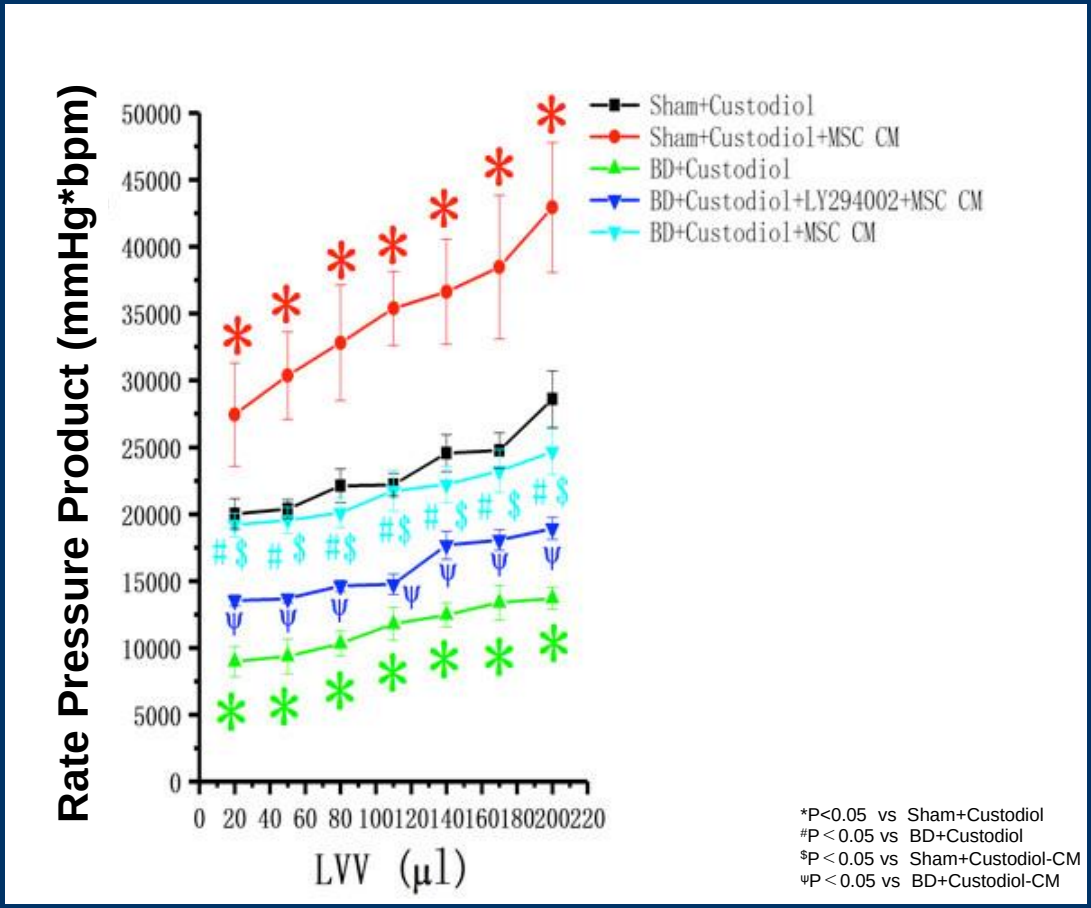


Significantly decreased LVSP, developed pressure, and dP/dt$_{max}$ were observed in the BD donor hearts when compared with the sham-operated group, indicating a decreased systolic function.

CM resulted in a better systolic functional recovery of grafts in both sham and BD groups.

Addition of LY294002 to the CM partially attenuated the protection afforded by CM in the BD group.

Effect of CM on myocardial work after transplantation – role of the PI3K pathway

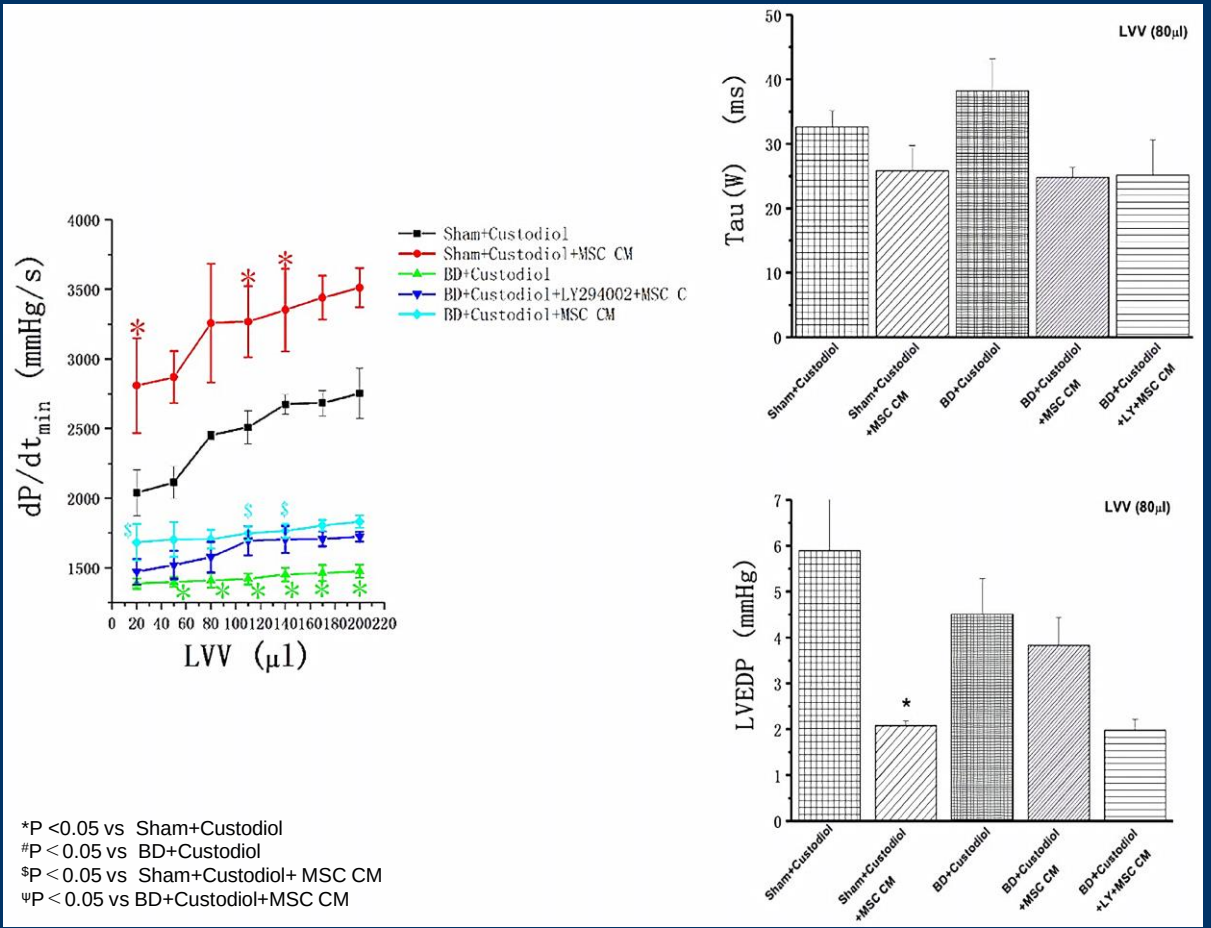


RPP was significantly decreased in the BD group when compared with the sham-operated group.

CM resulted in a better myocardial work recovery of grafts in both sham and BD groups as compared with their respective controls.

Addition of LY294002 to the CM significantly attenuated the improvement afforded by CM in the BD group.

Effect of MSC CM on LV diastolic function of the graft from BD donor hearts – role of the PI3K pathway

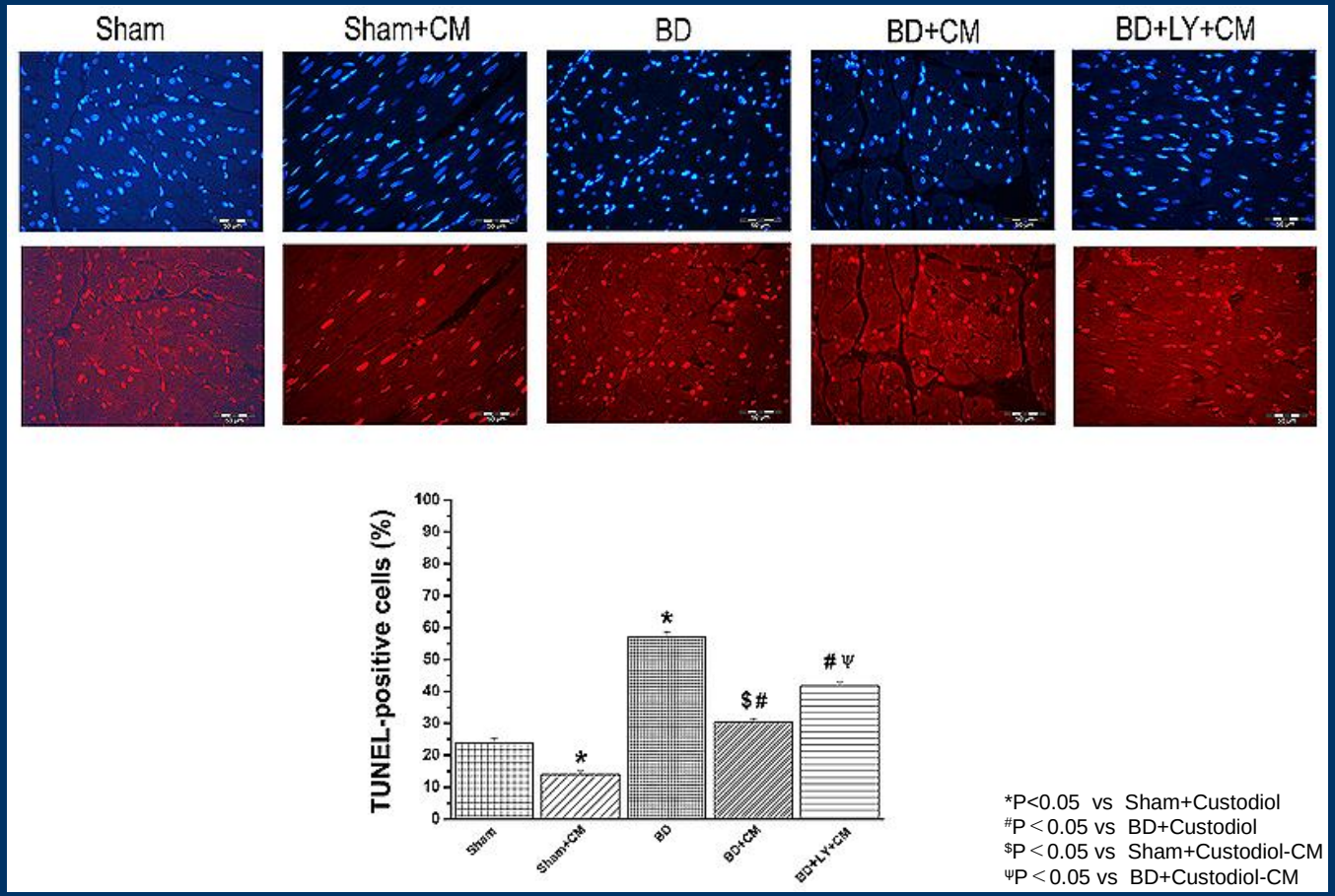


After transplantation, diastolic dysfunction was observed in the BD heart, as reflected by decreased dP/dt$_{min.}$

CM had no effect on diastolic dysfunction.

Addition of LY294002 to the CM had no effect.

DNA strand breaks of grafts – role of the PI3K pathway

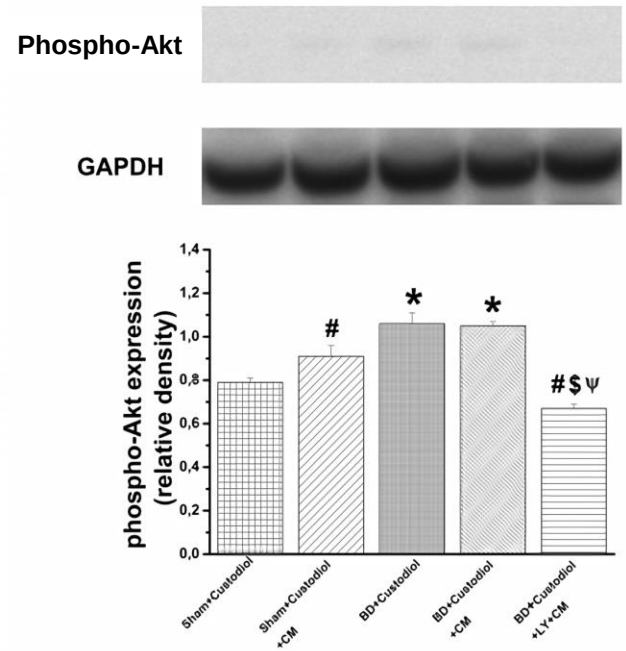


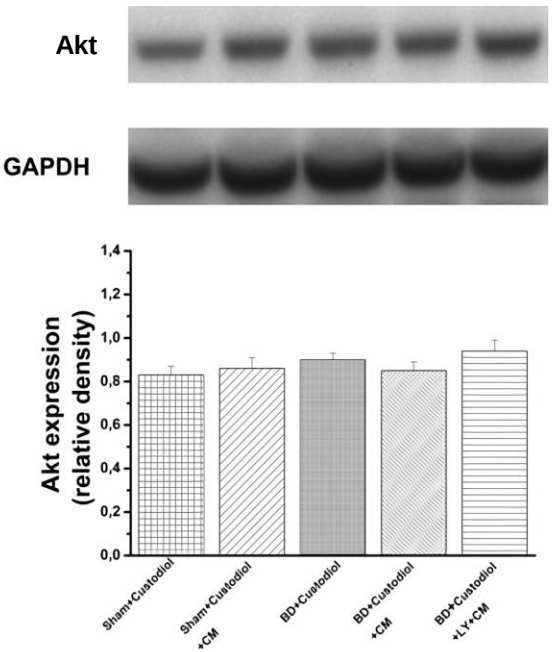
DNA fragmentation, as reflected by increased TUNEL positive cells, was observed in BD when compared with sham-operated group.

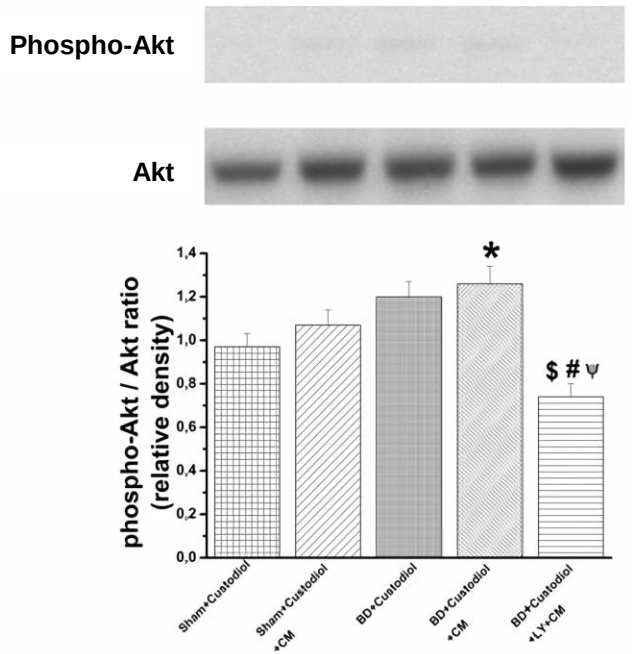
CM significantly reduced the DNA fragmentation of grafts in both sham and BD groups as compared with their respective controls.

Addition of LY294002 to the CM significantly attenuated the protection afforded by CM in the BD group.

Protein Expression of Grafts







*P<0.05 vs Sham+Custodiol
#P < 0.05 vs BD+Custodiol
$P < 0.05 vs Sham+Custodiol-CM
ΨP < 0.05 vs BD+Custodiol-CM

Western blot analysis demonstrated an increased expression of phosphorylated-Akt in BD group, however, total Akt and phosphorylated-Akt / Akt ratio were not changed.

CM treatment had no effect in expression of phosphorylated-Akt, total Akt, and phosphorylated-Akt /Akt ratio compared to their respective controls.

However, addition of LY to the CM significantly decreased expression of phosphorylated-AKT and phosphorylated-Akt / Akt ratio in BD group.

Conclusions

CM added to a preservation solution improves posttransplant cardiac contractility associated with brain death and ischemia/reperfusion injury.

Our data suggest that this protection is, partly, mediated by paracrine activation of the PI3K pathway.