Supplemental Material and Methods

Experimental model:

CPB causes systemic inflammatory response syndrome due to blood contact with non-endothelial surfaces. Both DHCA and reduced cerebral flow perfusion expose babies to cerebral reperfusion/reoxygenation under hypothermia. To reproduce unique and specific microenvironment resulting from complex pediatric cardiac surgery in the developing brain (i.e. hypothermic reperfusion/reoxygenation under inflammation), DHCA at 15^{o}C was performed for 60 minutes.

The gyrification of a 2 week old piglet cortex is similar to that of a human brain in the late 3rd trimester to early postnatal period[1]. We previously identified that the value of fractional anisotropy from diffusion tensor imaging in the same porcine white matter structures at postnatal 3 weeks was equivalent to those of human post-conceptual 41–53 weeks[2], indicating that the developmental time windows in the piglet model at this age correspond with the early neonatal period in the human brain. Based on our previous findings Yorkshire piglets at 2 weeks of age were used in the present study.

MSC development from human bone marrow:

Human MSCs were manufactured from bone marrow using the same methods that are used for clinical trials at the Good Manufacturing Practices clean room facility at Children's National Health System[3]. Initial samples were sent for aerobic, anaerobic, and fungal testing and were negative. Twenty-five mL of bone marrow was loaded into the Quantum Cell Expansion system after loaded the cell expansion set (disposable bioreactor) and coating it with 5 mg of fibronectin. After cells were allowed to adhere for 48 hours, cells were fed via perfusion D5 medium containing 5% human platelet lysate, 200 mM GlutaMAX, and 95% DMEM at a rate of 0.1 mL/min. Lactate was sampled daily, and once the lactate levels reached 4.0 mM, the feed rate was double until 4.0 mM was reached at 0.4 mL/min, at which point the cells were

harvested using TrypLE select, cryopreserved, and tested for phenotype, viability, and cell count. Twenty-million MSCs were then loaded into the Quantum for expansion. However, cells were cultured until the lactate levels were above 8 mM at 1.6 mL/min, at which point they were harvested. Cells were expanded in this manner for up to five total passages. With the exception of cells after the first passage, the majority of cells were cryopreserved until they were thawed, loaded into the Quantum, and used for subsequent passages. Prior to infusion, MSCs were tested according to the minimal criteria to define human MSC published by the International Society of Cellular Therapy[4]. This criteria includes >90% expression of CD73, CD90, CD105, and lack of expression of CD45, CD34, CD14 by flow cytometry, adipogenic, chondrogenic and osteogenic potential and plastic adherence. Cells were suspended in a vehicle composed of plasmalyte (85%), human serum albumin (5%) and dimethyl sulfoxide (DMSO) (10%). And they were stored in the vapor phase of liquid nitrogen until use. MSCs were thawed to room temperature and diluted with normal saline (total volume 10 ml). After 20 minutes of rewarming 10ml normal saline or MSCs (10×10^6 cells/kg) in normal saline were administered through the aortic cannula for 10 minutes according to the protocol. An average for the total number of MSCs was 36×10^6 cells.

Immunohistochemistry:

Brains were removed and further post-fixed at 4ºC in a 4% paraformaldehyde solution in 0.1 M PBS. Brains were cut into smaller tissue blocks. Tissue blocks were cryoprotected in a 15% sucrose solution for 24 hours, followed by a 48 hour incubation in a 30% sucrose solution in 0.1 M PBS at 4ºC. All samples were stored at -80^{o}C until immunohistochemical processing. Samples were embedded in O.C.T. compound, sliced with a cryostat at -20 ºC. Twenty-µm sections were incubated in blocking solution (20% normal goat serum, 1% bovine serum albumin, and 0.3% Tween 20 in phosphate buffered saline, pH 7.4) for 1 hour at room temperature. Sections were then incubated in primary antibodies, diluted in carrier solution (2%

normal goat serum, 2% bovine serum albumin, and 0.3% Tween 20 in phosphate buffered saline, pH 7.4), overnight at 4ºC. Species-specific, secondary fluorescent antibodies (1:500; Thermo Fisher scientific, Inc.) were diluted in carrier solution and applied to sections for 1 hour at room temperature. Sections were mounted with VECTASHIELD mounting medium for fluorescence with DAPI (Vector Laboratories, Inc.). The following primary antibodies were used for immunohistochemistry: anti-glial fibrillary acidic protein (GFAP) (1:500; Millipore sigma), anti-SOX2 (1:500; Millipore sigma), anti-Ki67 (1:500; BD Biosciences), anti-Doublecortin (Dcx) (1:500; Millipore sigma). Methodologies in BrdU labeling have not been established in the piglet model of DHCA: therefore Ki67 immunohistochemistry was used to determine cell proliferation. Cross reactivity of Ki67 antibody has been tested in the model[1,5]. Negative control was used for our cellular analysis.

Supplemental table 1. Experimental conditions

	Control (n=4)		DHCA (n=4)		DHCA+MSC (n=4)		
	Mean	SD	Mean	SD	Mean	SD	*P* value
Body weight (Kg)	4.6	0.5	4.4	1.0	3.6	0.5	0.124
Temperature (^{0}C)							
Pre CPB			37.2	0.8	36.3	0.7	0.112
End of CPB			36.9	1.7	36.5	1.1	0.713
3 hour CPB			35.6	1.7	35.6	0.7	0.959
SBP (mmHg)							
Pre CPB			83.0	10.1	79.0	16.8	0.689
End of CPB			122.5	9.0	131.4	23.1	0.496
3 hour CPB			77.8	23.8	87.0	10.0	0.501
Base excess (mmol/L)							
Pre CPB			4.9	3.0	4.7	2.2	0.912
End of CPB			-1.1	5.2	-2.9	2.9	0.593
3 hour CPB			-4.6	7.8	-8.2	5.5	0.551
Lactate (mmol/L)							
Pre CPB			1.3	0.7	1.5	0.4	0.619
End of CPB			6.0	0.5	8.7	3.4	0.240
3 hour CPB			6.6	4.8	8.2	6.5	0.747
Leukocyte (K/µL)							
Pre CPB			3.36	1.22	4.27	1.04	0.264
3 hour CPB			7.35	2.55	10.04	6.94	0.491
Hematocrit (%)							
Pre CPB			35.5	8.3	32.3	7.5	0.590
3 hour CPB			31.6	7.6	37.0	10.4	0.464
Total-Bilirubin (mg/dL)							
Pre CPB			0.5	0.2	0.8	0.3	0.163
3 hour CPB			1.1	0.7	1.1	0.3	1.000
BUN (mg/dL)							
Pre CPB			7.5	1.9	10.6	6.9	0.421
3 hour CPB			13.3	4.3	18.0	7.4	0.299
Creatinine (mg/dL)							
Pre CPB			0.78	0.12	0.76	0.17	0.842
3 hour CPB			0.89	0.23	0.98	0.21	0.549

DHCA, deep hypothermic circulatory arrest; MSC, mesenchymal stromal cell; CPB, cardiopulmonary bypass; SBP, systolic blood pressure; BUN, blood urea nitrogen; SD, standard deviation.

Supplemental figure legends

Figure Supplement 1. A, Study design. **B**, Surgical protocol. **C**, Sagittal plane of porcine brain. **D**, Coronal section from the boxed region in (C) and the subventricular zone (box). CPB, cardiopulmonary bypass; DHCA, deep hypothermic circulatory arrest; MSC, mesenchymal stromal cell; NT, normothermia; RW, rewarming.

Figure Supplement 2. A, $SOX2^+$ cells in SVZ. **B, C**, $SOX2^+Ki67^+$ cells in DL-SVZ (B), and V-SVZ (C). *P* values were determined by two-way ANOVA with Bonferroni comparisons. ***p*<0.01 vs. Control by unpaired Student's *t* test. Data are shown as mean ± standard error of mean (n=4 each). DHCA, deep hypothermic circulatory arrest; MSC, mesenchymal stromal cell; DL-SVZ, dorsolateral-subventricular zone; V-SVZ, ventral-subventricular zone.

Figure Supplement 3. A, Dcx^+ neuroblast number in tier 2 and 3 within the entire SVZ. **B**, Thickness of neuroblasts band in the entire SVZ. *P* values were determined by one-way ANOVA with Bonferroni comparisons. **p*<0.05 vs Control by unpaired Student's *t* test. Data are shown as mean ± standard error of mean (n=4 each). DHCA, deep hypothermic circulatory arrest; MSC, mesenchymal stromal cell; Dcx, doublecortin; SVZ, subventricular zone; F, F ratio in the ANOVA.

Figure Supplement 4. A, The size of Dcx^+ neuroblast clusters in SVZ. **B**, Ratio of total surface area of clusters to the entire SVZ. *P* values were determined by one-way ANOVA with Bonferroni comparisons. Data are shown as mean ± standard error of mean (n=4 each). DHCA, deep hypothermic circulatory arrest; MSC, mesenchymal stromal cell; Dcx, doublecortin; SVZ, subventricular zone; F, F ratio in the ANOVA.

Supplemental references

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