Published in final edited form as:

Ann Thorac Surg. 2010 June ; 89(6): 1965–1971. doi:10.1016/j.athoracsur.2010.02.051.

Transcriptional Profile of Brain Injury in Hypothermic Circulatory Arrest and Cardiopulmonary Bypass

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Abstract

Background—Little is known about the molecular mechanisms of neurologic complications after hypothermic circulatory arrest (HCA) with cardiopulmonary bypass (CPB). Canine genome sequencing allows profiling of genomic changes after HCA and CPB alone. We hypothesize that gene regulation will increase with increased severity of injury.

Methods—Dogs underwent 2-hour HCA at 18°C (n = 10), 1-hour HCA (n = 8), or 2-hour CPB at 32°C alone (n = 8). In each group, half were sacrificed at 8 hours and half at 24 hours after treatment. After neurologic scoring, brains were harvested for genomic analysis. Hippocampal RNA isolates were analyzed using canine oligonucleotide expression arrays containing 42,028 probes.

Results—Consistent with prior work, dogs that underwent 2-hour HCA experienced severe neurologic injury. One hour of HCA caused intermediate clinical damage. Cardiopulmonary bypass alone yielded normal clinical scores. Cardiopulmonary bypass, 1-hour HCA, and 2-hour HCA groups historically demonstrated increasing degrees of histopathologic damage (previously published). Exploratory analysis revealed differences in significantly regulated genes (false discovery rate < 10%, absolute fold change ≥ 1.2), with increases in differential gene expression with injury severity. At 8 hours and 24 hours after insult, 2-hour HCA dogs had 502 and 1,057 genes regulated, respectively; 1-hour HCA dogs had 179 and 56 genes regulated; and CPB alone dogs had 5 and 0 genes regulated.

Conclusions—Our genomic profile of canine brains after HCA and CPB revealed 1-hour and 2-hour HCA induced markedly increased gene regulation, in contrast to the minimal effect of CPB alone. This adds to the body of neurologic literature supporting the safety of CPB alone and the minimal effect of CPB on a normal brain, while illuminating genomic results of both.

Although more than 275,000 cardiac surgical procedures are performed annually, neurologic injury remains a troubling complication [1]. Injury severity ranges from stroke to subtle changes in neurocognition, leading to increased length of hospital stay, cost, and

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^{*}Recipient of the 2009 Hawley H. Seiler Resident Award.

Presented at the Basic Science Forum of the Fifty-sixth Annual Meeting of the Southern Thoracic Surgical Association, Marco Island, FL, Nov 4–7, 2009.

The Hawley H. Seiler Resident Award is presented annually to the resident with the oral presentation and manuscript deemed the best among those submitted for the competition. This Award was inaugurated in 1997 to honor Dr Seiler for his contributions and dedicated service to the Southern Thoracic Surgical Association.

rehabilitation needs, as well as decreased quality of life [2–4]. Estimates of postcardiac surgery cognitive dysfunction are as high as 25% at 3 months and 15% at 1 year [3,4].

Many studies have addressed potential neuroprotective strategies to prevent postcardiac surgery brain injury, but few have proven effective [5], suggesting the need for a greater understanding of the mechanism (s) of injury. A particularly important technique for neuroprotection in complex cardiac surgery is hypothermic circulatory arrest (HCA). Despite the widespread use of HCA, patients can experience significant neurologic injury, including seizures, stroke, developmental delays, and neurocognitive decline [6,7]. Experimental evidence has implicated post-HCA glutamate excitotoxicity as an important mediator of this injury [8].

Genomics and microarray technology hold tremendous potential to advance the practice of cardiac surgery. Some have advocated assessment of genetic polymorphisms preoperatively, which could impact patient responses to cardiopulmonary bypass (CPB) [9]. These technologies can assess gene regulation, identifying the extent to which genes are transcribed into RNA compared with baseline. If the change is statistically significant, that gene is said to be significantly regulated. Microarray technology has been used in patients [10] and experimental models [11] to investigate gene regulation in response to therapies and diseases. By yielding information regarding gene expression, genomics has the potential to yield new insights and confirm previous findings regarding the molecular mechanisms underlying postcardiac surgery brain injury.

Thus, gene expression in the hippocampus after HCA and CPB is investigated in an established experimental canine model. Previous work has identified the hippocampus as specifically injured during HCA [12]. Because the hippocampus is a center of neurocognition and memory [13], this study affords the potential to gain a greater mechanistic understanding of the subtle neurologic changes occurring after CPB and HCA. We hypothesize that the amount of gene regulation will increase with injury severity.

Material and Methods

Animals

For all experiments, we used a clinically relevant canine model of HCA and CPB, used in our laboratory for greater than 15 years [8,14–16]. Conditioned, heart-worm-negative, 6- to 12-month-old, 30-kg male class-A dogs were used for all experiments (Marshall Bioresources, North Rose, NY). Experiments were approved by The Johns Hopkins University School of Medicine Animal Care and Use Committee and complied with the “Guide for the Care and Use of Laboratory Animals” (1996, U.S. National Institutes of Health).

Experimental Design

Canine subjects were randomly assigned to 2 hours of HCA (2-hour HCA, $n = 10$), 1 hour of HCA (1-hour HCA, $n = 8$), or CPB alone ($n = 8$) and survived to either 8 hours ($n = 5$, 2-hour HCA; $n = 4$, 1-hour HCA and CPB) or 24 hours ($n = 5$, 2-hour HCA; $n = 4$, 1-hour HCA and CPB) after treatment. Additionally, 6 normal dogs, in which no interventions were performed, were sacrificed to serve as gene expression controls. The number of animals was chosen to adequately power microarray analyses after discussion with our microarray experts (C.C.T. and C.J.). Animals sacrificed 24 hours after treatment were neurologically scored using the Pittsburgh Veterinary Scoring System [17] before sacrifice. At the conclusion of the experiment, all subjects were sacrificed by exsanguination, and brains were harvested for analysis.

Detailed Procedures

SURGICAL HYPOTHERMIC CIRCULATORY ARREST PROCEDURE—Our experimental model has been described previously [8,14]. In brief, anesthesia is induced with sodium thiopental (25 mg/kg intravenous). The animals are endotracheally intubated and maintained on inhaled isoflurane (0.5% to 2%), 100% oxygen, and intravenous fentanyl (150 to 200 µg/dose). Probes are placed in the tympanic membrane (closely correlates with cerebral temperature), nasopharynx, and rectum to monitor temperatures throughout the experiment. A left femoral artery cannula is placed by means of open surgical cutdown for blood pressure monitoring and arterial blood gas sampling.

The CPB circuit consists of a membrane oxygenator with a 40-µm arterial filter (Cobe Laboratories, Lakewood, CO) and Sarns roller pump system (Sarns, Inc, Ann Arbor, MI). The circuit is primed with 1,500 mL of lactated Ringer's solution with potassium chloride (20 mEq). After heparinization (300 U/kg intravenous), the right femoral artery is cannulated (12F to 14F), and the cannula advanced into the descending thoracic aorta. Venous cannulas (18F to 20F) are advanced to the right atrium from the right femoral and right external jugular veins. All arteries and veins are accessed by means of open cutdown technique. Closed-chest CPB is initiated, and animals are cooled until tympanic membrane temperature reaches 18°C (approximately 30 minutes). Pump flows of 60 to 100 mL · kg⁻¹ · min⁻¹ are required to maintain a mean arterial pressure of 50 to 60 mm Hg, and activated clotting times are kept at greater than 500 seconds (every 15 minutes). Once tympanic temperatures reach 18°C, the pump is stopped and blood is drained by gravity into the reservoir.

Animals undergo 1-hour or 2-hour HCA with standard hemodilution and alpha-stat regulation of arterial blood gases (pH, 7.3 to 7.4; arterial partial pressure of oxygen > 300 mm Hg; arterial partial pressure of carbon dioxide, 30 to 40 mm Hg). Once HCA is complete, CPB is restarted, and rewarming (5°C temperature gradient every 15 minutes) to a core temperature of 37°C for 2 hours is performed. Warm intravenous fluids, a warm water blanket, and room temperature are used to maintain body temperature. Phenylephrine (intravenous, dosed to effect) is used to maintain mean arterial pressure greater than 75 mm Hg. If sinus rhythm does not return spontaneously, the heart is defibrillated at 32°C. At 37°C, animals are weaned from CPB, cannulas are removed, vessels are ligated, wounds are closed, and heparin is reversed with protamine (3 mg/kg intravenous).

Animals recover from anesthesia while intubated, with frequent monitoring of vital signs, arterial blood gases, and urine output. Once hemodynamically and clinically stable, they are extubated and transferred to their crate for recovery. Pain medication is administered per protocol after the procedure and at regular intervals.

CARDIOPULMONARY BYPASS—After induction and cannulation, animals undergo 2 hours of CPB with no HCA. Animals are cooled to 32°C, similar to routine clinical CPB. The heart continues to beat during this operation. Animals are recovered from anesthesia as described above.

NEUROLOGIC ASSESSMENT—The Pittsburgh Veterinary Score was assessed independently by 2 nonblinded study team members. The score includes 22 clinical questions relating to level of consciousness, respiration, cranial nerve function, reflexes, behavior, and motor and sensory function. No animals had neurologic impairment before experimentation, and no additional sedation was given within 12 hours before neurologic assessment.

SACRIFICE AND TISSUE PROCUREMENT—Animals are sacrificed by cold perfusion and exsanguination. They are sedated, intubated, and maintained on inhalational anesthesia. Median sternotomy is performed with ascending aortic cannulation (22F). The descending aorta is clamped, and CPB is initiated to ensure perfusion of the brain with 12 L of cold saline solution (4°C) at 60 mm Hg. The right atrial appendage is transected, and the venous return is vented. Brains are harvested by means of a large craniectomy, and the left hemisphere of the brain is immediately blocked and frozen (−80°C).

TISSUE PREPARATION—To ensure uniform sampling, the ventral anterior hippocampus was sectioned by cryostat at 20-μm intervals and a one in six series of sections (8 to 10 sections/sample, approximately 16 mg) was collected. Total RNA was isolated using the RNeasy Lipid Kit (Qiagen, Valencia, CA). RNA quality was assessed using a 2100 model Bioanalyzer (Agilent Technologies, Santa Clara, CA).

GENOMIC ANALYSIS—Canine microarray analysis was performed in a blinded fashion at the Johns Hopkins Microarray Core facilities using a single color strategy. The mRNA was reverse transcribed using oligo-DT primers, labeled with Cy3 (spectral peak, 532 nm), and normalized to a universal control mRNA. Arrays were fluorescence imaged with a confocal laser scanner to examine expression patterns within our mRNA samples [18]. Canine-specific long oligonucleotide (60-mer) expression arrays containing 44,000 features with 2,000 controls, representing approximately 42,000 unique transcripts, were used (Agilent Technologies, Santa Clara, CA) [19]. Oligomer (60-mer) probes were designed for open reading frames of *Canis familiaris* using OligoPicker [20] with GenBank and RefSeq as sources; BLAST-search against the *C familiaris* genome was carried out to ensure specificity. Gene expression profiles were compared between identical regions of canine brain (ventral anterior hippocampus) after HCA or CPB (compared with normal dogs).

MICROARRAY DATA ANALYSIS AND INTERPRETATION—Statistical analyses of microarray data were performed using the Bioconductor package *Affy* [21]; microarray signals were normalized using the quantile normalization method [22]. Exploratory data analysis was performed on these normalized data. To evaluate global changes in gene expression and examine expression patterns for genes of interest, volcano plots were constructed with probability values between conditions (Students' *t* test) on the *y* axis and log₂-fold changes on the *x* axis using Spotfire Decision Site for Functional Genomics (TIBCO Software Inc, Falls Church, VA). To screen for regulated genes and gene ontology (GO) terms, the false discovery rate was determined, with a false discovery rate of less than 0.10 and an absolute fold change of more than 1.2 considered significantly regulated [23]. Although the dog genome has been sequenced, it has not been fully annotated. To evaluate gene function, genes were related to orthologous human genes. The National Center for Biotechnology Information Entrez Gene database was used in conjunction with the dog genome database (University of California, Santa Cruz, CA). Entrez Gene identifications for human orthologs were assigned, and the Spotfire Gene Ontology Browser was used to determine GO biologic process terms regulated in each treatment group, compared with normal controls.

Statistical Analysis

Neurologic scores are presented as mean ± standard deviation. One-way analysis of variance was used to compare neurologic scores among groups, and post-hoc pairwise comparisons between groups were conducted using the Tukey-Kramer method. Statistical analysis was performed with the aid of STATA software (v9.2, Stata-Corp-LP, College Station, TX).

Results

Subjects

Ten dogs underwent 2-hour HCA, 8 had 1-hour HCA, and 8 had CPB alone. Half of the dogs in each group (5 2-hour HCA and 4 1-hour HCA and CPB) were sacrificed at 8 hours and half at 24 hours. Physiologic data ensuring the consistency of each technique are reported (Table 1). There were no operative or technical complications in any group.

Neurologic Scores

Using the neurologic scoring system (0 to 480), higher scores indicate worse neurologic function. Scores at 24 hours were significantly different among groups, with lower scores observed with decreasing severity of injury (Fig 1). For 2-hour HCA animals, the mean neurologic score was 182 ± 50 . In 1-hour HCA animals, the mean score was 58 ± 50 . In contrast to 2 1-hour HCA dogs that were effectively normal (scores < 20), no 2-hour HCA dog had a score less than 130—all were severely impaired. Cardiopulmonary bypass alone animals demonstrated no decrease in cognitive function (mean score, 8 ± 8).

Genomics

Microarray analysis revealed striking differences in the number of genes significantly regulated in each experimental group at each time (Table 2). Specifically, 2-hour HCA dogs had 502 and 1,057 genes regulated at 8 hours and 24 hours, respectively; 1-hour HCA dogs had 179 (8 hours) and 56 (24 hours) genes regulated; and CPB dogs had 5 (8 hours) and 0 (24 hours) genes regulated. Of these genes, 192 (2-hour HCA) and 17 (1-hour HCA) were regulated at both times. Figure 2 reveals notable overlap of regulated genes at 8 hours and 24 hours in 1-hour and 2-hour HCA groups. However, there was no overlap between genes regulated in CPB dogs with either HCA group at 24 hours; and only 2 overlapping genes between CPB and 1-hour HCA dogs at 8 hours. Highly regulated biologic process GO terms ($p < 0.005$) are shown in Table 2, and include “apoptosis,” “cell cycle,” and several immune system process terms, such as “inflammatory response” and “complement activation.” Genes within the “apoptosis” and “inflammatory response” GO terms are shown in Figures 3 and 4 as filled circles and triangles, respectively. Genes commonly regulated between the 1-hour and 2-hour HCA groups at 8 hours included six proapoptotic genes (*BCL2A1*, *GADD45B*, *GADD45A*, *TNFSF10*, *PLEKHF1*, and *PVR*), whereas commonly regulated genes at 24 hours included four antiapoptotic genes (*CCL2*, *HSPB1*, *ANXA1*, and *BNIP3*), as well as many genes mediating the inflammatory response. Some genes known to affect the apoptotic pathway were only regulated in the 2-hour HCA group (*BAG3*, *TP53*, and *STAT1*). As can be appreciated by examination of the volcano plots, the number of genes regulated differs considerably among treatment groups and correlates with both HCA time and observed degree of neurologic impairment.

Comment

This study examined canine subjects that underwent 2-hour HCA, 1-hour HCA, or CPB alone to determine the gene expression profiles within a particularly vulnerable area of the brain (hippocampus). The data show greater numbers of genes regulated after longer-duration HCA, which also produced clinically worse neurologic impairment. For example, dogs undergoing 2-hour HCA had increased numbers of genes regulated compared with 1-hour HCA, whereas dogs undergoing CPB alone had either no alterations in gene expression (24 hours) or minimal numbers of regulated genes (5 at 8 hours). This is notable given the high sensitivity of the relatively low absolute fold change significance requirement (≥ 1.2), the purpose of which was to cast a wide net for potentially regulated genes. These changes were consistent with the severity of clinical neurologic injury in each group. The lack of

significant gene regulation in CPB dogs is surprising, given this highly sensitive genomic analysis. This finding supports the contention that standard CPB has negligible long-term effects on a normal brain. This is consistent with our previous work investigating brain injury biomarkers after CPB and HCA, in which no change in levels of spectrin breakdown products were observed in CPB dogs, whereas there was a graded increase with longer durations of HCA [16].

Our 1-hour HCA model is meant to represent clinically relevant, yet prolonged, HCA duration. The 2-hour HCA model is purely experimental. It achieves a consistent severe neurologic injury (on pathologic examination, neurologic scoring, biomarker analysis, and genomic analysis), and is used to investigate potential therapeutics and compare findings in 1-hour HCA dogs. Examining the pattern of gene expression, 2-hour HCA dogs had more gene regulation at 24 hours than at 8 hours after injury, suggesting progression of injury with time. Conversely, in 1-hour HCA dogs, many genes regulated at 8 hours had returned to baseline by 24 hours (Fig 5). Coupled with the overlap in regulated genes in the two HCA groups, a pattern of gene regulation in HCA brain injury emerges, with proapoptotic genes regulated early and antiapoptotic and immune response genes regulated later (24 hours).

Examining the biologic process categories regulated in 1-hour HCA dogs yields insight into the cellular responses that are initiated in the brain after a clinically relevant duration of HCA. At 8 hours, the regulated genes are clustered in areas such as apoptosis, inflammatory response, defense response, response to wounding, and developmental processes. At 24 hours, fewer apoptotic and developmental genes are regulated. Additionally, there is increased activity in other immune system mechanisms, such as complement activation and cell motion. However, inflammatory and defense responses remain significantly regulated at both 8 and 24 hours after HCA. Although 10% to 20% of regulated genes in each group were regulated at both times, further elucidation of important pathways is necessary to identify their significance.

It appears that the brain's response to HCA in the first 24 hours is marked by early programmed cell death and initiation of developmental processes, late immune system and complement activation, and regulation of the inflammatory and defensive responses. These findings are interesting when taken in the context of specific genes that are regulated and have been previously reported to influence brain injury after HCA.

Prior Work

Previous animal studies have reported the hippocampus to be particularly vulnerable to injury after HCA [8,12,15,24,25]. Research in animal models and patients have suggested a regulation of inflammatory and apoptotic genes after CPB and HCA [24,26]. However, our study reports the transcriptional profiling of animals undergoing HCA and CPB. Microassays have been previously used to study changes in gene expression after HCA and CPB in rodents [25]. That genes of known importance in the pathway of glutamate excitotoxicity, mitochondrial injury, and early apoptosis are regulated in our study helps to support and extend our previous investigations on the importance of this pathway for the post-HCA brain [27].

Two studies of interest involve the expression of apoptotic genes in models of HCA and CPB. Ananiadou and colleagues [24] demonstrated increased levels of the anti-apoptotic mitochondrial protein *BCL2* within porcine hippocampus 3 hours after 75 minutes of 18°C HCA. This is consistent with our observations that apoptotic genes are significantly regulated after clinically relevant HCA. Zhang and colleagues [28] also implicated regional increases in apoptotic mitochondrial proteins *BCL2* and *BAX* in the hippocampus up to 6 hours after hypothermic CPB in a rodent model. Among the five *BCL2* family members

examined in our study, BCL2A1 was most highly regulated; with fourfold to fivefold increased expression at both times after 2-hour HCA and at 8 hours after 1-hour HCA.

This study builds on previous work on brain injury after HCA and CPB from our laboratory and others by applying the emerging technologies of canine genomics in our established model. This transcriptional profiling of the 24 hours after these procedures affirms the importance of inflammatory and apoptotic processes in the response of the brain to HCA. This characterization of transcriptional regulation holds promise to identify potential therapeutic targets and greater mechanistic understanding as the specifics of the genes involved and their interactions are more fully elucidated.

Limitations

Our study is limited by our small sample size and limited information on the temporal pattern of gene expression. Our goal was to focus on short-term changes in gene expression and to begin defining the temporal relationships of expression in different biologic process GO terms. We acknowledge the current study provides little information on long-term changes in gene expression. Additionally, we recognize that the effects of CPB and HCA in comorbid adult patients undergoing cardiac surgery could differ from those in young and healthy canine subjects. However, the molecular mechanisms and gene regulation after HCA-induced injury are likely similar. Our experimental design is further limited by lack of control groups receiving 18°C CBP alone (without HCA) or anesthesia alone. Hence, we cannot comment on the effect of arrest beyond hypothermia on brain injury or the impact of anesthesia alone on gene expression. As well, our 1-hour HCA duration is longer than routine clinical HCA, but represents the upper limit of acceptable clinical HCA duration.

Conclusions

Brain injury after cardiac surgery remains a significant clinical concern, the origin of which is not completely understood. Genomics provides a window into the transcriptional profile of the brain after HCA and CPB. In this initial analysis, CPB leads to minimal changes in gene regulation, whereas HCA results in significant regulation of several genes, including those affecting apoptosis and inflammation. The magnitude of these alterations in gene expression increases with duration of HCA.

Acknowledgments

This study was supported by the Dana and Albert Broccoli Center for Aortic Diseases, the Mildred and Carmont Blitz Cardiac Research Fund, and the National Institutes of Health (NIH R37NS31238-10WAB, NIH 2T32DK007713-12ESW). Doctor Allen is the Hugh R. Sharp Cardiac Surgery Research Fellow, and Drs Weiss and Arnaoutakis are the Irene Piccinini Investigators in Cardiac Surgery. The authors wish to thank Jeffrey Brawn, Melissa Jones, Tamara Treat, and Jennifer Berrong for their outstanding technical assistance.

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Fig 1.

Mean neurologic scores based on University of Pittsburgh Canine Neurological Score (0–480) 24 hours after 2-hour hypothermic circulatory arrest (2H HCA), 1-hour hypothermic circulatory arrest (1H HCA), or cardiopulmonary bypass (CPB) alone. Higher score indicates worse neurologic function. Error bars are one standard deviation. * $p < 0.05$ by Tukey-Kramer method after one-way analysis of variance (ANOVA).

Fig 2.

Venn diagram demonstrating the number of genes significantly regulated within each treatment group and the number of coregulated gene in the three groups at 8 (A) and 24 (B) hours. (CPB = cardiopulmonary bypass; 1h HCA = 1 hour of hypothermic circulatory arrest; 2h HCA = 2 hours of hypothermic circulatory arrest.)

Fig 3.

Volcano plots of mRNA expression in the hippocampus of dogs 8 hours after 2-hour hypothermic circulatory arrest (2h HCA; A), 1-hour hypothermic circulatory arrest (1h HCA; B), or cardiopulmonary bypass (CPB; C). Symbols indicate biologic process gene ontology term: filled circle = apoptosis; filled triangle = inflammatory response; star = apoptosis and inflammatory response; cross = other/no annotation. Genes in black meet our statistical criteria: false discovery rate < 0.10 and absolute fold change ≥ 1.2 .

Fig 4.

Volcano plots of mRNA expression in the hippocampus of dogs 24 hours after 2-hour hypothermic circulatory arrest (2h HCA; A), 1-hour hypothermic circulatory arrest (1h HCA; B), or cardiopulmonary bypass (CPB; C). Symbols indicate biologic process gene ontology terms: filled circle = apoptosis; filled triangle = inflammatory response; star = apoptosis and inflammatory response; cross = other/no gene ontology annotation. Genes in black meet our statistical criteria: false discovery rate < 0.10 and absolute fold change ≥ 1.2 .

Fig 5.

Summary plot of the temporal pattern of gene expression in each treatment group with notable overlapping gene ontology categories regulated at each time. (CPB = cardiopulmonary bypass; 1H HCA = 1 hour of hypothermic circulatory arrest; 2H HCA = 2 hours of hypothermic circulatory arrest.)

Table 1

Mean Physiologic and Technical Data for Experimental Procedures

Variable	2-Hour HCA (N = 10)	1-Hour HCA (N = 8)	CPB (N = 8)
Baseline Hb (mg/dL)	11.6 ± 0.9	11 ± 1	12.0 ± 0.9
Lowest tympanic temperature (°C)	17.7 ± 0.8	18.4 ± 0.2	30.9 ± 0.8
Time to HCA (min)	32 ± 3	26 ± 6	...
Time from HCA to 37°C (min)	108 ± 6	107 ± 10	...
Hb during HCA/CPB (mg/dL)	10 ± 1	9 ± 1	8.6 ± 0.7
Hb after HCA/CPB (mg/dL)	9.6 ± 0.7	9 ± 1	8.1 ± 0.7

CPB = cardiopulmonary bypass; Hb = hemoglobin; HCA = hypothermic circulatory arrest.

Numbers of Significantly Regulated Genes at 8 and 24 Hours After 2-Hour Hypothermic Circulatory Arrest, 1-Hour Hypothermic Circulatory Arrest, and Cardiopulmonary Bypass Alone^a

Table 2

Gene Ontology Term	Genes in GO Term ^b	2-Hour HCA		1-Hour HCA		CPB	
		8 h Survival	24 h Survival	8 h Survival	24 h Survival	8 h Survival	24 h Survival
Biologic process (all terms)	14,221	502	1057	179	56	5	0
Negative regulation of cellular biosynthetic process	465	32	52	8	1	1	0
Developmental process	3120	125	278	59	17	2	0
Apoptosis	897	30	100	23	7	0	0
Inflammatory response	265	8	36	10	5	0	0
Defense response	605	18	68	16	12	0	0
Response to wounding	346	12	51	15	8	0	0
Immune system process	975	34	134	17	13	0	0
Complement activation	40	3	11	0	3	0	0
Cell motion	480	19	58	8	10	0	0
Cell cycle	842	32	84	12	5	1	0
Circulatory system process	196	10	30	5	1	0	0
Hemopoiesis	255	14	36	6	2	0	0
Chemotaxis	159	4	19	4	5	0	0
Extracellular matrix organization	74	3	7	4	4	1	0

^aOverall number of genes and those within specific GO categories are reported that met a false discovery rate < 0.1 and an absolute fold change ≥ 1.2.

^bTotal number of genes annotated with the indicated GO term. Italicized numbers indicate GO terms significantly regulated for each treatment group ($p < 0.005$).

CPB = cardiopulmonary bypass; GO = gene ontology; HCA = hypothermic circulatory arrest.