Cardioplegia prevents ischemia-induced transcriptional alterations of cytoprotective genes in rat hearts: A DNA microarray study

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Background: Energy conservation and calcium homeostasis contribute to myocardial protection provided by hyperkalemic cardioplegia during ischemia. Complementing these established mechanisms of protection, previous work suggested that activation of cytoprotective signaling pathways also contributes to reduced injury with cardioplegia. We proposed that cardioplegia would recruit cytoprotective pathways and investigated the transcriptional response of the heart after cardioplegia-protected ischemia compared with that after ischemia alone.

Methods: Isolated perfused rat hearts underwent 40 minutes of global ischemia alone or with St Thomas cardioplegia, followed by 120 minutes of reperfusion. The expression profiles of isolated RNA were determined by using Affymetrix microarrays and assessed by comparing cardioplegia-protected hearts and hearts undergoing unprotected ischemia with time-matched control hearts. The content of selected proteins was assessed by means of immunoblotting.

Results: Cardioplegia preserved the expression of multiple genes involved in carbohydrate and fatty acid metabolism, glycolysis, and electron transport compared with ischemia alone. The expression of the sodium-calcium exchanger and ryanodine receptor was preserved in line with the ability of cardioplegia to decrease calcium overload. The expression of multiple cytoprotective molecules, including protein-tyrosine kinase, calcineurin B, p38 mitogen-activated protein kinase, voltage-dependent anion channel, protein kinase C ϵ , heat shock protein 70, and manganese superoxide dismutase all showed decreased expression in ischemia but were preserved to near nonischemic levels by cardioplegia.

Conclusion: Cardioplegia during ischemia maintained an expression profile similar to that seen in nonischemic hearts for genes involved in energy conservation, calcium homeostasis, and cytoprotective pathways, whereas ischemia alone did not. Exposing the transcriptional differences in cytoprotective genes during untreated and cardioplegia-treated ischemia provides valuable insight into an additional mechanism of cardioprotection induced by cardioplegia.

Cardioplegic arrest is used to provide myocardial protection in the majority of the 686,000 patients who undergo cardiac surgical procedures in the United States each year.¹ The arrested heart, although advantageous to the surgeon, remains susceptible to ischemic injury. Hyperkalemic cardioplegia (CP) is used to maintain diastolic arrest and provide myocardial protection by attenuating the progression of ischemic injury. Despite modifications to the formulation and route of administration, suboptimal myocardial protection remains a problem. The protective effect of CP is thought to be largely the result of conservation of cellular energy stores and preservation of calcium homeostasis. In addition to these accepted mechanisms of myocardial protection, previous work from our laboratory demon-

strated that the myocardial protection provided by CP is substantially attenuated if intracellular signaling pathways involving protein kinase C (PKC) or tyrosine kinase are inhibited.² Activation of these signaling kinases leads to cytoprotection and has been implicated in the robust cardioprotective response of ischemic preconditioning (IPC). This evolving appreciation of the key contribution of the activation of intracellular signaling pathways to myocardial protection³ raises the question of whether similar mechanisms might contribute to the cardioprotection provided by CP. To identify potential key contributing mechanisms involved in the cardioprotection of CP, we used DNA microarray technology to profile the gene expression pattern of rat hearts subjected to ischemia and reperfusion alone compared with that seen in hearts subjected to CP-protected ischemia and reperfusion. The goal of this study was to evaluate transcriptional changes in genes related to previously known mechanisms of CP and to assess for evidence of the novel involvement of cytoprotective pathways.

Materials and Methods

All experiments were approved by the Case Western Reserve University Institutional Animal Care and Use Committee and conformed to the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council and published by the National Academy Press (revised 1996).

Isolated Rat Heart Preparation

The isolated rat heart preparation was used as previously described.² Male Sprague-Dawley rats (320–360 g) were anesthetized (heparin, 1000 units/kg administered intraperitoneally; pentobarbital, 100 mg/kg administered intraperitoneally). Hearts were excised, mounted on the Langendorff apparatus, and perfused with Krebs-Henseleit (KH) buffer delivered at a perfusion pressure of 65 mm Hg. After collection of baseline data during the equilibration period, the hearts were randomized into one of the 3 study groups. Time-matched control hearts ($n = 7$) were perfused for 180 minutes. Hearts in the ischemic group ($n = 7$) were equilibrated for 20 minutes and then underwent 40 minutes of normothermic global ischemia, followed by 120 minutes of reperfusion with KH buffer. Hearts in the CP group ($n = 7$) were equilibrated for 17 minutes, perfused with St Thomas' Cardioplegia II (NaCl, 110.0 mmol/L; KCl, 16.0 mmol/L; CaCl₂, 1.20 mmol/L; MgCl₂·6H₂O, 16.0 mmol/L; and NaHCO₃, 10.0 mmol/L; pH of 7.8 ± 0.2 , $T = 4^{\circ}\text{C}$) for 3 minutes, and underwent 40 minutes of normothermic global ischemia, followed by 120 minutes of reperfusion with KH buffer. After 180 minutes, left ventricular (LV) tissue was excised and immediately frozen in liquid nitrogen. Three hearts from each group were used for RNA preparation, and 4 hearts were used independently for protein quantitation.

Microarray Analysis

Samples of total RNA were processed by the Gene Expression Array Core Facility at Case Western Reserve University.

RNA preparation. Total RNA was isolated from LV tissue by using Trizol Reagent (Invitrogen). RNA preparations from each group (ischemic, CP, and control) were pooled ($n = 3$), and their quality and quantity (0.79, 0.91, and 1.10 μg RNA/mg LV tissue) were assessed with spectrophotometry (260/280) and gel electrophoresis. Samples were cleaned and eluted by using a column from a Qiagen RNeasy kit (part no. 74106) precipitated with ammonium acetate and ethanol centrifuged, washed, and resuspended.

Synthesis of cDNA. The Affymetrix protocol for cDNA synthesis was used. The reaction was primed by annealing an oligo-dT primer coupled to a T₇ RNA polymerase promoter to the RNA sample. RNA was reverse transcribed with Superscript II reverse transcriptase. Second-strand synthesis was carried out immediately in the presence of *Escherichia coli* DNA pol I, RNase H, and DNA ligase; incubated in the presence of T₄ DNA pol; and terminated with ethylenediamine tetraacetic acid (EDTA). The sample was cleaned (Qiagen cDNA clean-up column).

Synthesis of biotin-labeled complementary RNA. Complementary RNA was generated in an in vitro transcription reaction by using a BioArray High Yield ENZO kit (Affymetrix). Samples were mixed, centrifuged, and returned to the incubator every 40 minutes. In vitro transcription samples were cleaned (Qiagen RNA clean-up columns) and eluted. The quality was confirmed spectrophotometrically (260/280).

Fragmentation and hybridization to test array. Samples were fragmented (40 mmol/L Tris acetate [pH 8.1], 100 mmol/L potassium acetate, and 30 mmol/L magnesium acetate at 94°C for 35 minutes) and added to hybridization buffer (final concentrations: 100 mmol/L morpholinoethanesulfonic acid (MES); 1 mol/L [Na⁺]; 20 mmol/L EDTA; 0.01% Tween 20; 0.1 mg/mL Herring sperm DNA; and 0.5 mg/mL acetylated bovine serum albumin) to improve hybridization to the oligonucleotide array. Eukaryotic hybridization controls (*BioB*, *BioC*, *BioD*, and *cre*; final concentrations of 1.5, 5, 25, and 100 pmol/L, respectively) and control oligonucleotide (50 pmol/L) were added to the cocktail. The hybridization cocktail was denatured (99°C for 5 minutes), transferred to the test array, and incubated (42°C for 16 hours), and the hybridization cocktail was removed. The array was washed, stained, and scanned for background fluorescence and expression levels of control oligonucleotides by using Affymetrix Microarray Suite software.

Hybridization of fragmented samples to species microarray. Sample hybridization cocktails were thawed (45°C) and centrifuged. The Affymetrix chip arrays (RG-U34A, part no. 510338), containing probe sets for more than 7000 known genes, were equilibrated to room temperature. Sample cocktails were introduced into the chamber of the preconditioned chips and incubated (45°C for 16 hours). The chips were washed, stained, and scanned with an Agilent Gene Array scanner 2000 driven by Affymetrix MicroArray Suite 5.0. Genes with unknown products or duplicate entries were omitted. Functional or biologic classification was determined from the Affymetrix database, the PubMed database, or both.

Statistical analysis of species microarray. Scanned images were analyzed with Affymetrix MAS 5.0 software. For complete description of the statistical algorithms, refer to the Statistical Algorithm Description Document available from Affymetrix. The following tunable parameters were used: $\alpha 1 = 0.04$ and $\alpha 2 =$

0.06 for single-array analysis, and $\tau = 0.015$, $\gamma_{1L} = 0.0025$, $\gamma_{1H} = 0.0025$, $\gamma_{2L} = 0.003$, and $\gamma_{2H} = 0.003$ for comparison analysis. Statistical significance is determined by query of 3 parameters: detection, fold change, and change. To be regarded as increased in the treated sample compared with the control, detection was "P" (present) or "M" (marginal), fold change was 2 or more, and change was "I" (increased). For decreases, detection in the sample was "P" or "M," fold change was -2 or less, and change was D (decreased). The change algorithm uses the Wilcoxon signed-rank test with corresponding cutoffs of γ ($P < .0025$ vs control).

Western Analysis

Frozen ventricular tissue was placed in buffer (Tris-HCl, 50 mmol/L [pH 7.4]; sodium orthovanadate, 0.1 mmol/L; sodium fluoride, 50 mmol/L; sucrose, 150 mmol/L; phenylmethylsulfonyl fluoride, 1 mmol/L; EDTA, 5 mmol/L; ethyleneglycol-bis-(β -aminoethylether)-N,N,N',N'-tetraacetic acid, 2 mmol/L; 0.2% Triton X-100, Sigma protease inhibitor cocktail 1:200 [producing final concentrations of 4-(2-aminoethyl)benzenesulfonyl fluoride HCl (AEBSF), 500 μ mol/L; Aprotinin, 0.4 μ mol/L; Leupeptin, 10 μ mol/L; Bestatin, 18 μ mol/L; Pepstatin, 7.5 μ mol/L; E-64, 7 μ mol/L]) and homogenized (Polytron, 2 bursts of 30 seconds, speed 5). The lysate was mixed on ice for 60 minutes. The solubilized proteins were centrifuged at 360g for 10 minutes, and the resulting supernatant was assayed for total protein concentration by using the Bradford method. Total protein (15 μ g) was separated by means of sodium dodecylsulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membrane. Nonspecific binding was blocked with 5% nonfat milk, and the membrane was incubated with primary antibody (anti-p38, mouse monoclonal sc-7972, Santa Cruz Biotechnology Inc; anti-protein-tyrosine kinase [anti-JAK2], rabbit polyclonal, Upstate Biotechnology; anti-sodium-calcium exchanger, rabbit polyclonal, Swant). The membrane was washed and incubated with the appropriate horseradish peroxidase-linked secondary antibody, washed, and then incubated with a chemiluminescent agent (Luminol, Santa Cruz Biotechnology). Photographic film was exposed to the chemiluminescence, and the bands were quantified by means of densitometry (Scion Image, Scion Corp) as normalized to a control lysate.

Statistical Analysis of Left Ventricular Developed Pressure Recovery, Lactate Dehydrogenase Release, and Protein Content

Data are expressed as means $\pm$ SEM. One-way analysis of variance with the Tukey post-hoc test were performed on left ventricular developed pressure (LVDP) recovery, lactate dehydrogenase (LDH) release, and protein content.

Results

LVDP and LDH

Time-matched control hearts performed as expected for this model, with preserved LVDP after 180 minutes. Ischemic hearts showed decreased recovery of function compared with control hearts. CP treatment before ischemia markedly protected against the decrease in LVDP (Figure 1). Ischemic

Figure 1. Recovery of function (top) at 180 minutes and LDH release during minutes 60 through 90. LVDP, Left ventricular developed pressure; LDH, lactate dehydrogenase. * $P < .001$ versus control or cardioplegia groups.

hearts showed a significant increase in LDH release compared with that seen in nonischemic control hearts, as well as compared with hearts undergoing CP-treated ischemia (Figure 1). Values for LVDP and LDH release are comparable with those obtained previously in this model.²

Expression Profile

The gene expression profile of rat hearts subjected to CP-protected ischemia and reperfusion was compared with the profile in hearts subjected to ischemia and reperfusion alone and with the time control group. The number of genes with 2-fold or greater change was reduced by removing probes with unknown products and duplicates (multiple probe sets that hybridize to the same product). After this reduction,

TABLE 1. Biologic function of genes with decreased expression after ischemia but preserved with cardioplegia

No. of genes	Function/description
Energy/metabolism	
2	Acetyl CoA synthesis from pyruvate
1	ATP metabolism
7	Carbohydrate metabolism
5	Electron transport/energy production
7	FA metabolism
7	Other
Calcium regulation	
2	Calcium regulation
1	Calcium channel binding
2	Calcium binding
Immune/inflammatory	
4	Immune/inflammatory
Cytoprotective-ischemia/IPC/stress	
4	Stress response
3	Glutathione synthesis/oxidative stress
3	Heat shock/chaperone
2	Oxidative stress
5	Other
Transcription	
11	Transcription
Other	
9	Protein transport
4	Adhesion molecule
4	Angiogenesis
4	Cytoskeletal
2	Mg ²⁺ -dependent phosphatase
3	Growth
2	Cell cycle
19	Other

A complete list is available online in Table E2. *CoA*, Coenzyme A; *ATP*, adenosine triphosphate; *FA*, fatty acid; *IPC*, ischemic preconditioning.

146 genes were expressed with a 2-fold or greater change in the unprotected ischemic group that was unchanged in the CP-protected ischemic group compared with the nonischemic control group. With the same 2-fold cutoff point, 18 differences were found to be unique to the CP-treated group but not to the ischemia group compared with the control group. Thirty-three genes demonstrated 2-fold or greater change in both the ischemia and CP-treated groups compared with the control group. Most of the changes unique to the ischemic group demonstrated decreased expression (112/146), whereas changes unique to CP treatment predominantly reflected increased expression (11/18).

Genes with increased expression in the ischemic group but not in the CP-treated group represent products that display a diverse array of functions (Table E1) and include some potentially related to cardioprotection. Among the genes exhibiting decreased expression after ischemia but

that were not diminished with CP protection (Tables 1 and E2) are 29 genes involved in carbohydrate metabolism, fatty acid metabolism, glycolysis, electron transport, or other cellular processes involved in energy production. Five genes involved in calcium regulation or calcium binding, including the sodium-calcium exchanger (NCx) and ryanodine receptor, had decreased expression after ischemia unless protected with CP. Genes for proteins involved in cytoprotection, including JAK2, calcineurin B, p38 mitogen-activated protein kinase (MAPK), voltage-dependent anion channel, PKC-binding protein enigma, PKC- ϵ , heat shock protein 70-related gene and precursor, and mitochondrial manganese superoxide dismutase, also did not maintain baseline levels of their transcripts after ischemia unless first protected with CP.

Genes exhibiting decreased expression in both the ischemia and CP-treated groups were predominantly those that function in the inflammatory or immune responses, notably tumor necrosis factor α and interleukin 1 (Table E3). The genes with increased expression after ischemia in both the untreated and CP-treated groups compared with the control group were few and included heat shock protein 70 (Table E3).

Of the few genes altered by CP-protected ischemia, 3 related to the immune-inflammatory response, including interleukin 18, demonstrated decreased expression (Table 2). Genes with increased expression in CP treatment but not ischemia displayed seemingly diverse functions, with the largest change being an increase in a heat shock protein (Table 2).

Content of Selected Proteins

Protein content was evaluated by means of Western analysis and compared with transcript levels to validate selected results observed at the level of gene translation. Western analysis revealed a significantly reduced content of p38 MAPK in untreated ischemic hearts compared with either control ($P < .001$) or CP-treated hearts ($P < .001$, Figure 2). The protein content of JAK2 was also reduced in ischemic hearts compared with control hearts ($P = .009$) or hearts undergoing CP-treated ischemia ($P = .019$). Differences between groups in NCx protein content, however, were not significant (data not shown).

Discussion

This study was designed to investigate potential mechanisms whereby CP provides myocardial protection by evaluating changes that occur at the level of transcription. Relative transcript abundance was evaluated by using the microarray approach. The alterations in gene expression caused by ischemia alone or ischemia protected with CP was assessed in isolated rat hearts perfused by means of the Langendorff method.

TABLE 2. Changed expression with cardioplegia but not ischemia

Gene no.	Ischemia vs C	CP vs C	Gene product	Function-description
Immune-inflammatory				
U65217	−1.2	−3.5	MHC class II antigen RT1.B beta	Immune/inflammatory
U77777	NC	−2.1	Interleukin 18	Immune/inflammatory
X54250	−1.9	−2.0	HIV type I enhancer-binding protein 1	Immune/inflammatory
Cytoprotective-ischemia/IPC/stress				
U53922	1.7	2.3	DnaJ-like protein (RDJ1)	Heat shock/chaperone
AA108277	1.5	5.7	similar to Heat shock protein (hsp-E7I)	Heat shock/chaperone
Transcription				
L20681	NC	2.0	ETS-1 proto-oncogene	Transcription
D26307	−1.7	2.1	jun-D	Transcription
Other				
D12769	1.6	2.1	Kruppel-like factor 9	Growth and development
X06769	1.9	2.3	c-fos	Growth and development
U75397	1.9	2.3	Krox-24	Growth and development
AF034899	NC	−2.5	Olfactory receptor-like (SCR D-9)	Chemoreceptor
AF063102	−1.2	−2.3	CIRL-2	α -Latrotoxin receptor
D10757	−1.5	−2.3	Proteasome subunit, β -9	Protein catabolism
L35558	−1.7	−2.1	Glutamate/aspartate transporter	Glutamate signaling
U49099	1.9	2.0	Cis-Golgi p28 (p28)	Protein transport
M94918	1.7	2.0	Hemoglobin beta chain	Oxygen transport
Y13275	NC	2.0	Transmembrane 4 family member 3	Blood coagulation
AF030088	NC	2.0	Homer	Glutamate signaling

Values represent fold change. Negative designates decrease. C, Control; CP, cardioplegia; MHC, major histocompatibility complex; NC, no change; CIRL-2, calcium-independent receptor of alpha-latrotoxin-2.

The Langendorff model chosen, using 40 minutes of normothermic global ischemia, eliminates the confounding effects of hypothermic ischemia and was shown to provide good resolution between treatments in functional end points (Figure 1).² Microarray analysis in the 3 groups revealed differences corroborating the known protective mechanisms of CP, namely energy conservation and calcium homeostasis. The transcriptional data also support the preservation of a nonischemic phenotype by cardioplegia, a new observation.

It is important to recall that although collectively ischemia is damaging to cardiac function, the changes in the expression of individual genes is not necessarily detrimental. Likewise, although CP protection improves function, each of the changes in individual gene expression levels is not necessarily beneficial. The stimulus of ischemia or CP might evoke a combination of responses activating adaptive or protective mechanisms, as well as disrupting normal cell functions. A comparison of gene expression levels between ischemia and CP-protected ischemia therefore might provide clues as to the mechanism of cardioprotection, as well as its limitations. Additionally, gene expression studies are not definitive but are rather preliminary studies to focus further investigation.

The greatest disruption to the normal transcription process occurred in the untreated ischemic group, with 133 genes demonstrating a 2-fold or greater decrease in expres-

sion. CP treatment limited the decreased transcript content to merely 28 genes. The ability of CP treatment to maintain expression levels nearer to control for genes otherwise altered by ischemia suggests that CP, in general, preserves a nonischemic transcriptional profile and is likely to maintain a nonischemic phenotype. The protein content data shown in Figure 2 support this notion.

The ability of CP treatment to maintain transcription levels of genes involved in carbohydrate metabolism, fatty acid metabolism, glycolysis, electron transport, and calcium homeostasis suggests mechanisms of CP involving the relative conservation of energy and prevention of cellular calcium overload. The NCx and ryanodine receptor are important regulators of Ca^{2+} homeostasis. Increased expression of NCx and ryanodine receptor has been demonstrated as an adaptive response to low-flow ischemia.⁴ Although we were unable to demonstrate differences in the protein content for NCx, here we show increased gene expression in CP-protected ischemia compared with that in ischemia alone for these important regulators of calcium homeostasis.

Assessing the changes that occur to gene expression by cardioplegia protection but not by ischemia alone (Table 2) arguably would appear most pertinent to the mechanism of cardioplegia. Surprisingly, this category contained relatively few genes. Furthermore, the magnitude of change was small, with just one gene exhibiting greater than a 3-fold

Figure 2. Myocardial abundance of p38 mitogen-activated protein kinase (*p38*, top) and protein-tyrosine kinase (*JAK2*, bottom) in time-matched control (open bar), ischemic (hatched bar), or cardioplegia-treated (filled bar) hearts after 2 hours of reperfusion or equivalent time for control hearts ($n = 4$). Units are relative to a common, nonischemic heart value set at 1.00. Western blotting shows representative hearts ($n = 2$) for each group. * $P < .05$ compared with control or cardioplegia-treated hearts.

increase and one with a greater than 3-fold decrease. This finding refutes our hypothesis that cardioplegia would activate numerous cytoprotective pathways but rather suggests that the cardioprotection of cardioplegia might be less a factor of transcriptional changes induced by the treatment

Figure 3. Cardioplegia preserves expression of genes with cytoprotective function. Open bars represent unprotected ischemia, and filled bars represent cardioplegia-protected ischemia. Data are displayed relative to control values of 1, such that a 2-fold decrease (-2) is equivalent to 0.5. *HSP70*, 70-kd Heat shock protein; *KATP-1*, adenosine triphosphate-sensitive potassium channel uKATP-1; *MAPK P-1*, mitogen-activated protein kinase phosphatase 1; *HSP70 precursor*, 70-kd heat shock protein precursor; *MAP3K8*, serine-threonine protein kinase (Map3k8); *PKC-ε*, protein kinase C ϵ ; *MnSOD*, manganese superoxide dismutase; *Heme Ox*, heme oxygenase; *HSP-related (hst70)*, heat shock protein-related gene hst70; *SCS-3*, suppressor of cytokine signaling 3; *VDAC*, voltage-dependent anion channel; *p38 MAPK*, p38 mitogen-activated protein kinase; *JAK2*, protein-tyrosine kinase.

and more a function of minimizing ischemia-induced alterations in transcription.

Of particular focus in this study were changes in genes known to be involved in cytoprotective mechanisms, including IPC. As with other cluster groups, CP-protected ischemia produced expression levels closer to baseline levels than did unprotected ischemia for genes related to cytoprotective pathways (Figure 3). Interestingly, several genes with decreased expression in ischemia but preserved with CP treatment have previously been shown to have roles in the cardioprotective response during ischemia and reperfusion (Tables 1 and E2). These include JAK2,⁶ calcineurin B,^{7,8} p38 MAPK,⁹ voltage-dependent anion channel,³ PKC-binding protein enigma,¹⁰ PKC- ϵ ,¹¹ heat shock protein 70-related gene and precursor,¹² and manganese superoxide dismutase.¹³ Although the precise role for each of these gene products is unclear, the evidence implicating them in ischemia or cardioprotective mechanisms makes the alterations observed in our study intriguing. Repressed gene expression of these mediators after ischemia, as we have

demonstrated for p38 and JAK2 (Figure 2), might indicate the cell's inability to maintain appropriate content of these peptides and thus contribute to injury. The ability of the CP-protected heart to maintain expression levels much closer to the baseline levels suggests mechanisms of cardioplegia-induced protection that were previously unknown.

Genes with increased expression in ischemia but not CP compared with control values (Table E1) included the adenosine triphosphate–sensitive potassium channel and MAPK phosphatase 1, proteins involved in modulation of cardiac injury during ischemia. Once thought to be the final step in the signaling cascade of IPC,⁵ the opening of mitochondrial adenosine triphosphate–sensitive potassium channels is one of many important alterations in mitochondrial proteins that might contribute to cardioprotection.³ Exactly which members of the MAPK cascades are involved and how they function remains unclear. However, much evidence supports their involvement in the cardioprotection of IPC.^{3,5} Increased expression after ischemia of proteins related to these mechanisms could be indicative of a response by the cell to upregulate their production in an attempt to attenuate ischemic damage. If this upregulation is beneficial and yet deficient in the CP-treated group, this finding might provide an opportunity to improve the protection provided by CP. To validate the transcriptional changes for select genes and to assess the potential physiologic effect, we quantified 3 proteins of interest. The protein content of p38 MAPK and JAK2 correlated with the observed change in transcription (Figure 2). The smaller change in transcript levels of NCx was not reflected in protein content (data not shown). Further characterization of these changes by measuring abundance of specific transcripts and protein activity might be beneficial to determine their role in cardioprotection.

Also noteworthy is the disproportionate number of genes involved in the inflammatory response, which displayed decreased expression in ischemia and CP (Table E3). Recognizing the relationship of ischemia to oxidative stress and of the latter to inflammation, we anticipated increased expression for many inflammatory markers in the ischemic group but not in the CP group. Perhaps this represents the extent of dysfunction caused in ischemia and in CP treatment before ischemia or occurs because of the use of the buffer-perfused heart as the model of experimental ischemia. This finding nonetheless suggests that the heightened inflammatory response prevalent after cardiopulmonary bypass (CPB) might not occur as a result of CP but rather is activated by other factors. The unique expression profile in human atrial tissue after CPB and cardioplegic arrest¹⁴ provides valuable data to support this statement. These investigators noted increased expression of inflammatory genes after CPB. Our observation of predominantly decreased inflammatory genes in the isolated perfused heart

suggests that the increases observed in the clinical setting might occur because of one or more of the additional factors associated with the clinical cardiac surgical setting, including perhaps the CPB circuit, hypothermia, surgical trauma, anesthesia, or preexisting ischemia. Additionally, induced genes unique to the clinical study might be reflective of differences between analysis of LV tissue from an isolated rat heart and atrial tissue from CPB surgery or merely limitations of the microarray technique. Future studies will be needed to delineate these differences.

The current study evaluated differences in the gene expression pattern between hearts subjected to ischemia alone or hearts protected with hyperkalemic CP before ischemia. Ischemia altered the pattern of expression to a much greater extent in untreated hearts than in hearts first protected with CP, suggesting that CP-protected ischemia tends to preserve the nonischemic transcriptional phenotype. Many of the genes exhibiting decreased expression caused by ischemia alone are important in energy production and calcium homeostasis, which is consistent with proposed mechanisms of CP-induced myocardial protection. In addition, the cardioprotection provided by CP maintains cytoprotective peptides reminiscent of IPC. The novel concept that cardioplegia can prevent ischemic disruptions to the transcriptional processes of many genes, including those involved in cytoprotection, is worthy of consideration for advancing myocardial protective strategies during cardiac surgery.

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TABLE E1. Increased expression with ischemia but unchanged with cardioplegia

Gene no.	Ischemia vs C	CP vs C	Gene product	Function/description
Energy/metabolism				
D16102	5.7	NC	ATP-stimulated glucocorticoid-receptor transl promoter	Carbohydrate metabolism
M33648	2.6	NC	3-Hydroxy-3-methylglutaryl-CoA synthase	Acetyl CoA metabolism
U07971	2.5	1.7	L-Arginine-glycine amidinotransferase	Amino acid metabolism
S69874	2.1	NC	Cutaneous fatty acid-binding protein	FA metabolism
X51529	2.0	NC	Platelet phospholipase A ₂	FA metabolism
L46791	2.5	NC	Cholesterol esterase	Carboxylesterase activity
Calcium regulation/contractility				
D29646	2.0	NC	ADP-ribosyl cyclase (CD38)	Calcium regulation
X52840	2.3	NC	Myosin regulatory light chain	Contractility
Immune/Inflammatory				
L23128	2.1	1.7	MHC class I	Immune/inflammatory
M64986	2.0	1.2	Amphoterin	Immune/inflammatory
Cytoprotective-ischemia/IPC/stress				
D42145	3.0	1.5	ATP-sensitive potassium channel uKATP-1	Stress/IPC/cardioprotection
S81478	2.6	NC	MAP kinase phosphatase 1	Stress induced
L01702	2.6	1.6	Protein-tyrosine-phosphatase (LRP)	PKC-dependent Src act
K01932	2.1	NC	glutathione S-transferase Yc subunit	Glutathione synthesis/stress
Transcription				
Z17223	3.0	1.9	Gax protein	Transcription
AF000942	2.6	1.4	Id3a	Transcription
X61296	2.5	NC	L1 retroposon, ORF2	Transcription
U17254	2.5	NC	Immediate early gene transcription factor NGFI-B	Transcription
U91679	2.1	NC	ETS domain transcription factor Pet-1	Transcription
Other				
X59859	2.3	NC	decorin	Extracellular matrix org
Y13714	2.0	NC	osteonectin	Extracellular matrix org
U75928	2.0	1.3	SPARC	Extracellular matrix org
J04035	2.0	1.4	Tropoelastin	Extracellular matrix org
X55183	2.8	1.9	Schwannoma-derived growth factor	Growth factor
M81183	2.3	1.4	Insulin-like growth factor I	Growth factor
M31837	2.0	NC	Insulin-like growth factor-binding protein (IGF-BP3)	Growth factor
J05495	2.5	1.6	Gal/GalNAc-specific lectin	Binds to Gal/GalNAc
U82623	3.0	NC	RaIA binding protein 1	Protein transport
M96601	2.3	NC	Taurine transporter	Neuroprotective
D14014	2.3	NC	Cyclin D1	Cell-cycle regulation
X71127	2.1	1.3	Complement protein C1q β -chain	Complement activation
V01227	2.0	1.4	α -Tubulin	Microtubule movement
U42627	2.0	NC	Dual-specificity protein tyrosine phosphatase (rVH6)	Phosphatase
X12957	2.6	NC	γ Crystallin gene cluster	Eye lens proteins

Values are fold changes. C, Control; CP, cardioplegia; NC, no change; ATP, adenosine triphosphate; CoA, coenzyme A; FA, fatty acid; ADP, adenosine diphosphate; MHC, major histocompatibility complex; IPC, ischemic preconditioning; MAP, mitogen-activated protein; PKC, protein kinase C; ORF2, open reading frame 2; NGFI-B, nerve growth factor-induced clone B; SPARC, Secreted Protein Acidic and Rich in Cysteine.

TABLE E2. Decreased expression after ischemia but preserved with cardioplegia

Gene no.	Ischemia vs C	CP vs C	Gene product	Function/description
Energy/metabolism				
AF034577	−3.7	NC	Pyruvate dehydrogenase kinase (PDK4)	Acetyl CoA synthesis from pyruvate
Z12158	−3	NC	PDH E1 α form 1 subunit	Acetyl CoA synthesis from pyruvate
D13376	−2.5	−1.3	Adenylate kinase 1	ATP metabolism
M27726	−4.9	NC	Phosphorylase (B-GP1)	Carbohydrate metabolism
U20643	−4	−1.3	Aldolase A	Carbohydrate metabolism
AFFX-h	−3.5	NC	Brain hexokinase	Carbohydrate metabolism
D26393	−3.5	−1.6	Type II hexokinase	Carbohydrate metabolism
U20195	−3.2	NC	Phosphoglucomutase (Pgm1)	Carbohydrate metabolism
M93401	−2.1	NC	Methylmalonate semialdehyde DH	Carbohydrate metabolism
S50461	−2.1	NC	G protein α 12 subunit	Carbohydrate metabolism
D10952	−3.5	NC	Cytochrome c oxidase subunit Vb	Electron transport/energy production
AA686031	−3.5	NC	Like NADH-ubiquinone oxidoreductase 75	Electron transport/energy production
M21476	−2.5	NC	Iodothyronine 5-monodeiodinase (5-MD)	Electron transport/energy production
AA685112	−2.5	NC	Like NADH-ubiquinone oxidoreductase 23	Electron transport/energy production
M24542	−2.1	NC	Rieske iron-sulfur protein	Electron transport/energy production
Y17295	−3	NC	1-Cys peroxiredoxin	FA metabolism
AB005743	−2.8	NC	CD36 antigen	FA metabolism
AB010429	−2.6	NC	Acyl-CoA hydrolase-like protein	FA metabolism
X98225	−2.6	NC	Gastrin-binding protein	FA metabolism
AF047707	−2.6	−1.3	UDP-glucose:ceramide glycosyltransferase	FA metabolism
AF063302	−2.3	NC	Carnitine palmitoyltransferase I β	FA metabolism
E03344	−2.1	NC	Peroxisome-forming factor	FA metabolism
D44481	−2.3	NC	Crk II adaptor protein	Glucose transport/adaptor protein
D10853	−2.3	NC	Amidophosphoribosyltransferase	Glutamine metabolism
L01793	−2.3	NC	Glycogenin	Glycogen synthesis
D21869	−2.6	NC	Phosphofructokinase-M	Glycolysis
J05571	−2	NC	S-adenosylmethionine synthetase	Metabolism
M58364	−2.3	−1.9	GTP cyclohydrolase I	Synthesis of cofactor BH4
AF034577	−3.7	NC	Pyruvate dehydrogenase kinase (PDK4)	Acetyl CoA synthesis from pyruvate
Calcium regulation				
X68191	−3.5	NC	Sodium-calcium exchanger	Calcium regulation
U95157	−2.5	NC	Ryanodine receptor type II (RyR2)	Calcium regulation
U48247	−3.2	NC	Protein kinase C-binding protein enigma	Calcium channel binding
L18889	−2.3	NC	Calnexin	Calcium binding
X53363	−2.1	NC	Calreticulin	Calcium binding
Immune/inflammatory				
U45965	−3.5	−1.9	Inflammatory protein-2 precursor	Immune/inflammatory
X17053	−3.0	−1.9	Immediate-early serum-responsive JE gene	Immune/inflammatory
M92340	−2.0	−1.2	Interleukin 6 signal transducer	Immune/inflammatory

TABLE E2. continued

Gene no.	Ischemia vs C	CP vs C	Gene product	Function/description
AF029240	−2.0	−1.9	MHC class Ib RT1.S3 (RT1.S3)	Immune/inflammatory
Cytoprotective-ischemia/IPC/stress				
M94454	−2.1	NC	Serine-threonine protein kinase (Map3k8)	Stress response
U13396	−9.2	−1.5	Protein-tyrosine kinase (JAK2)	Stress response
U91847	−7.5	NC	p38 Mitogen-activated protein kinase	Stress response
M18331	−2.3	−1.9	Protein kinase C ϵ	Stress response
J05181	−7	−1.4	γ -Glutamylcysteine synthetase	Glutathione synthesis/oxidative stress
X02904	−2.3	−1.4	Glutathione S-transferase P subunit	Glutathione synthesis/oxidative stress
J03914	−2	−1.3	Glutathione S-transferase Yb subunit	Glutathione synthesis/oxidative stress
X15705	−2.8	NC	Heat shock protein-related gene hst70	Heat shock/chaperone
AA848268	−2.3	NC	Similar to DnaJ protein homolog 2	Heat shock/chaperone
S75280	−2	NC	70-kd Heat shock protein precursor	Heat shock/chaperone
Y00497	−2.3	NC	MnSoD	Oxidative stress
U63923	−2.3	NC	Thioredoxin reductase	Oxidative stress
D14568	−8	NC	Calcineurin B	Ischemia
AF075383	−2.8	NC	Suppressor of cytokine signaling 3	Jak/STAT pathway
AJ005984	−2.8	−1.5	α -Endosulfine	K(ATP) channel regulation
AF048828	−3.5	NC	Voltage-dependent anion channel (VDAC)	Mitochondrial calcium transport/IPC
J02722	−2.5	NC	Heme oxygenase	Cleaves heme/stress induced
Transcription				
M65251	−3.5	−1.9	Hivep2	Transcription
X54686	−2.6	−1.4	pJunB	Transcription
X54249	−2.5	−1.6	Zinc finger protein AT-BP1	Transcription
M65149	−2.3	−1.7	CELF	Transcription
D37951	−2.3	−1.5	MIBP1 (c-myc intron binding protein 1)	Transcription
M55017	−2.3	NC	Nucleolin	Transcription
X63369	−2.3	−1.5	Zinc finger protein Tis 11	Transcription
AF009330	−2.0	NC	SHARP-2	Transcription
L14462	−2.0	NC	R-esp1	Transcription
L14463	−2.0	NC	R-esp2	Transcription
U51584	−2.0	NC	Zinc finger enhancer-binding protein 2	Transcription
Other				
X53565	−7.0	NC	Trans-Golgi network protein TGN38	Protein transport
U64689	−4.3	NC	Synaptotagmin interacting protein zygin II	Protein transport
X12535	−2.6	NC	Rab7	Protein transport
AF052596	−2.3	NC	SNAP-23	Protein transport
M83676	−2.1	NC	Rab12	Protein transport
X13905	−2.1	NC	Ras-related rab1B protein	Protein transport
AF019043	−2.0	NC	Dynamin-like protein (DLP1)	Protein transport
X92097	−2.0	NC	Coated vesicle membrane protein	Protein transport

TABLE E2. continued

Gene no.	Ischemia vs C	CP vs C	Gene product	Function/description
AF072935	-2.0	NC	Rab5	Protein transport
AF097593	-2.6	NC	N-cadherin	Adhesion molecule
L23088	-2.1	-1.5	P-selectin	Adhesion molecule
AF097593	-2.0	NC	Testicular N-cadherin	Adhesion molecule
M84488	-2.5	-1.5	Vascular cell adhesion molecule 1	Adhesion molecule
L20913	-3.5	NC	Vascular endothelial growth factor form 3	Angiogenesis
X14232	-2.5	NC	Heparin-binding growth factor 1	Angiogenesis
M32167	-2.3	NC	Glioma-derived VEGF	Angiogenesis
U93306	-2.5	NC	VEGF receptor-2/FLK-1	Angiogenesis
AF004811	-4.0	NC	Moesin	Cytoskeletal
AF053768	-3.2	NC	Cortactin-binding protein CBP90	Cytoskeletal
AFFX-ba	-2.8	NC	Cytoplasmic β -actin	Cytoskeletal
AFFX	-2.1	NC	β -Actin	Cytoskeletal
S74572	-3.0	NC	Mg ²⁺ -dependent protein phosphatase β	Mg ²⁺ -dependent phosphatase
S90449	-3.0	NC	Protein phosphatase 2C isoform	Mg ²⁺ -dependent phosphatase
AF071204	-4.0	-1.7	Kit ligand 1 precursor	Stem cell factor
M11794	-3.7	-1.9	Metallothionein 2 and metallothionein 1	Zinc homeostasis
AJ223083	-12.1	NC	Retinoic acid X receptor γ -1	Growth and development
M25350	-3.2	NC	cAMP phosphodiesterase (PDE4) s	cAMP-mediated pathways
S77528	-3.0	NC	C/EBP-related transcription factor	Apoptosis
AB000362	-3.0	NC	Cold-inducible RNA-binding protein	Cold response
U77829	-3.0	-1.4	Gas-5 growth arrest homolog	Growth arrest
X60212	-2.8	NC	Ribosomal protein L17	Translation
L13407	-2.6	NC	CaM kinase II δ subunit	Smooth muscle cell migration
U75920	-2.5	-1.6	APC-binding protein EB1	Cell cycle/microtubule binding
D45247	-2.5	-1.2	Proteasome subunit RCX	Ubiquitin protein catabolism
D16308	-2.3	-1.6	Cyclin D2	Cell cycle
M58040	-2.3	-1.4	Transferrin receptor	Iron transport
D90404	-2.1	NC	Cathepsin C	Protease
Z83757	-2.1	NC	Growth hormone receptor	Growth
X16043	-2.1	NC	Phosphatase 2A catalytic subunit α	Phosphatase
D00092	-2.0	NC	70-kd mitochondrial autoantigen	Autoantigen
X77934	-2.0	NC	Amyloid precursor-like protein 2	Amyloid secretion
X99337	-2.0	NC	Glycoprotein 55	Glycoprotein
E05646	-2.0	-1.2	Hippocampal choline neuropeptide HCNP	Phosphatidyl ethanolamine binding
U61373	-2.0	NC	Proteinase-activated receptor 2	G protein signal transduction
D84477	-2.0	NC	RhoA	Ras-related/growth factor binding
M74494	-2.0	1.2	Sodium/potassium ATPase α -1 subunit	Na/K regulation
U50842	-2.0	-1.3	Ubiquitin ligase (Nedd4)	Protein modification

Values represent fold change. Negative designates decrease. C, Control; CP, cardioplegia; NC, no change; CoA, coenzyme A; PDH, pyruvate dehydrogenase; ATP, adenosine triphosphate; DH, dehydrogenase; NADH, nicotinamide adenine dinucleotide; FA, fatty acid; UDP, uridine diphosphate; GTP, guanine triphosphate; BH4, tetrahydrobiopterin; MHC, major histocompatibility complex; IPC, ischemic preconditioning; MnSoD, manganese superoxide dismutase; K(ATP), ATP-sensitive potassium channel; AT-BP1, antitrypsin binding protein 1; CELF, CUG-BP and ETR-3 like factor; SHARP-2, split- and hairy-related protein-2; SNAP-23, synaptosomal-associated protein-23 kD; VEGF, vascular endothelial growth factor; cAMP, cyclic adenosine monophosphate; APC, adenomatous polyposis coli; ATPase, adenosine triphosphatase.

TABLE E3. Changed expression with both ischemia and cardioplegia

Gene no.	Ischemia vs C	CP vs C	Gene product	Function/description
Energy/metabolism				
D87240	−5.3	−4.6	Fructose-6-phosphate 2-kinase	Carbohydrate metabolism
AF047707	−2.0	−2.0	UDP-glucose:GlcT	Lipid transport and metabolism
Immune/inflammatory				
U17035	−7.5	−5.3	mob-1	Inflammatory/immune response
E02468	−7.0	−2.1	TNF	Inflammatory/immune response
M80367	−6.1	−2.6	Guanylate-binding protein 2	Inflammatory/immune response
Y07704	−5.3	−2.8	BEST5	Inflammatory/immune response
M98820	−4.3	−4.0	Interleukin 1 β	Inflammatory/immune response
M34253	−4.3	−2.6	IRF-1	Inflammatory/immune response
D00403	−4.0	−3.0	Interleukin 1 α	Inflammatory/immune response
U68272	−4.0	−2.3	Interferon γ receptor	Inflammatory/immune response
U06434	−3.7	−2.6	MIP-1 β	Inflammatory/immune response
AF030358	−3.7	−2.3	Chemokine CX3C	Inflammatory/immune response
X52711	−3.5	−4.0	Mx1 protein	Inflammatory/immune response
X63594	−3.5	−2.6	RL/IF-1	Inflammatory/immune response
L00981	−3.2	−2.1	TNF- β , TNF- α	Inflammatory/immune response
U22414	−2.3	−2.1	Inflammatory protein-1 α	Inflammatory/immune response
Cytoprotective-ischemia/IPC/stress				
AF055714	4.6	2.3	Hypertension-reg vasc factor-1C-4	Stress response
L16764	4.6	4.9	Heat shock protein 70 (HSP70)	Stress response
Transcription				
U18314	3.0	2.3	Thymopoietin	Transcription
AF015953	3.2	2.1	aryl hydrcrbn recept nucl transctr	Transcription
L23148	9.8	3.5	Inhibitor of DNA binding 1	Transcription, angiogenesis
Other				
L2552	−7.0	−2.6	E-selectin (ELAM-1)	Adhesion molecule
D00913	−4.3	−2.1	Intercellular adhesion molecule 1	Adhesion molecule
X63722	−3.2	−2.0	Vascular cell adhesion molecule 1	Adhesion molecule
AJ011969	−3.5	−2.8	Growth differentiation factor 15	TGF- β signaling
X57523	−2.8	−2.1	mtp1	Iron transport
AFFXbbm	2.0	2.0	7,8-Diamino-pelargonic acid	Biotin synthesis proteins
U17604	3.0	2.1	Reticulon 1	Endocytosis
U78102	3.5	2.3	krox20	Growth and development
L26292	4.6	3.7	FSH-regulated protein	Follicle-stimulating hormone related
AF036548	4.6	3.7	RGC-32	Response gene to complement 32
M26686	5.3	4.0	Carboxyl methyltransferase	Protein modification
L19998	7.0	4.0	Minoxidil sulfotransferase	Steroid metabolism

Values represent fold change. Negative designates decrease. *C*, Control; *CP*, cardioplegia; *UDP*, uridine diphosphate; *TNF*, tumor necrosis factor; *BEST5*, bone-expressed sequence tag 5; *IRF-1*, interferon regulatory factor-1; *MIP-1 β* , macrophage inflammatory protein-1 β ; *RL/IF-1*, nuclear factor of dappa light chain gene enhancer in B-cells inhibitor, alpha; *FSH*, follicle stimulating hormone; *RGC-23*, response gene to complement 32.