Moderate hypothermia during cardiopulmonary bypass increases intramyocardial synthesis of heat shock protein 72

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Objectives: This study was undertaken to test the hypothesis that the myocardial protective effect of moderate hypothermia during cardiopulmonary bypass involves upward regulation of heat shock protein 72.

Methods: Sixteen young pigs were randomly assigned to a temperature regimen during standardized cardiopulmonary bypass of normothermia or moderate hypothermia (temperatures 37°C and 28°C, respectively, n = 8 per group). Myocardial probes were sequentially sampled from the right ventricle before and during bypass and 6 hours after bypass. Messenger RNA encoding for heat shock protein 72 was assessed by competitive reverse transcriptase–polymerase chain reaction, and heat shock protein 72 synthesis was assessed by Western blot and immunohistochemical methods. Induction of apoptosis was assessed by gene expression of apoptosis-regulating proteins (Bcl-xL, Bak, and Fas) according to competitive reverse transcriptase polymerase chain reaction. Apoptotic cells were identified with an in situ apoptosis-detection kit (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling) in combination with morphologic criteria. Necrotic cells were detected by standard histologic methods.

Results: Moderate hypothermia rather than normothermia was associated with earlier and higher gene expression and synthesis of heat shock protein 72 in the myocardium during and after cardiac surgery. In the hypothermia group both heat shock protein 72 and the messenger RNA encoding it were detected as soon as 30 minutes after initiation of bypass and before aortic clamping, whereas in the normothermia group they were not detected before aortic clamping. Immunohistochemical methods showed localization of heat shock protein 72 in the cardiomyocytes, endothelial cells, and macrophages. Although the percentage of necrotic cells in the myocardium was lower in the hypothermic group, the induction of apoptosis regulatory proteins and the percentage of apoptotic cells did not differ between the groups.

Conclusions: These results suggest that the myocardial protective effect of moderate hypothermia during cardiopulmonary bypass involves upward regulation of heat shock protein 72 and inhibition of necrosis but not of apoptosis.

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In a recent experimental study, we showed that moderate hypothermia during cardiopulmonary bypass (CPB) attenuates the systemic inflammatory reaction associated with cardiac surgery and provides organ protection by inducing an anti-inflammatory cytokine balance.¹ In this respect, the myocardium of an animal subjected to moderately hypothermic CPB shows less histologic and ultrastructural signs of cell damage and cell death than does that of a control animal operated on under normothermia.² The mechanisms controlling cell damage and cell death are complex. They involve natural protective processes that repress the production of proinflammatory and harmful proteins and control cell death by inhibiting the necrosis or the apoptosis pathways.³ Both apoptotic and necrotic forms of cell death are induced by death domain receptors. Whereas necrosis is strongly inflammatory and devastating to neighboring tissues, however, apoptosis is a noninflammatory, self-regulating process that could limit tissue damage and contribute to the removal of activated inflammatory cells.^{4,5} Apoptosis is related to the expression of the surface receptor Fas⁶ and activation of the caspase pathway,^{7,8} which is controlled by the Bcl-2 protein family of both apoptosis-inhibiting (Bcl-xL) and apoptosis-promoting proteins (Bak).

A well-known mechanism providing cellular protection is the induction of heat shock proteins (HSPs). HSPs are molecular chaperones expressed after cellular insult⁹ that exert a protective effect by refolding damaged proteins.⁹ Experimental studies suggest that HSP-70 could participate in the control of apoptosis by refolding damaged repressors of the apoptotic pathway.¹⁰ Inducers of the heat shock response include, among others, heat treatment and inflammatory mediators.¹¹ HSP-70 is the member of the HSP family that has been most extensively studied, and HSP-72, its inducible form, is exclusively expressed during stress.¹² A recent *in vitro* study has suggested that HSP-70 is also induced by cold,¹³ but to date the question of whether hypothermic CPB would induce HSP-72 expression has not been addressed. This experimental study was designed to test the hypothesis that the myocardial protective effect of moderate hypothermia during CPB is related to upward regulation of HSP-72.

Material and Methods

This study was conducted according to the guidelines of the German Animal Protection Law ensuring humane care and was approved by the supervising state agency for animal experiments. Sixteen female pigs weighing 38 ± 0.5 kg (mean $\pm$ SEM) were randomly assigned to a temperature group during CPB ($n = 8$ per group): normothermia, with a temperature of 37°C, and moderate hypothermia, with a temperature of 28°C.

General anesthesia was uniform and consisted of a combination of ketamine and pentobarbital given in repeated doses as necessary. After endotracheal intubation, the lungs of the animals were

mechanically ventilated with an air and oxygen mixture (inspired oxygen fraction 0.5). Core temperature was monitored with an esophageal temperature probe (probe 16561; Datex-Ohmeda Division, Instrumentarium Corp, Helsinki, Finland). Cefotiam (50 mg/kg) was given intravenously for antibiotic prophylaxis. Catheters were placed in the carotid artery and jugular vein as well as in the left atrium.

CPB equipment was uniform and consisted of a roller pump inducing a nonpulsatile flow, a disposable pediatric hollow-fiber oxygenator, a hard-shell cardiomy reservoir, an arterial blood filter, and a bubble trap. The extracorporeal perfusion circuit was primed with a crystalloid solution. Bovine lung heparin (400 IU/kg) was given for systemic anticoagulation. Both caval veins, the aorta, and the left atrium were cannulated, allowing the institution of total CPB. Total duration of CPB was set at 120 minutes for all animals; this included 30 minutes of perfusion during which animals operated on in moderate hypothermia were cooled down to 28°C with a heat exchanger, 60 minutes of perfusion during which the aorta was crossclamped, and 30 minutes of perfusion during which pigs undergoing hypothermic CPB were rewarmed to 37°C. In the normothermia group, perfusion was performed at 37°C. CPB was conducted with a flow index of 2.7 L/(min $\cdot$ m² body surface area) in all animals, with a target mean systemic arterial pressure of 60 mm Hg. Immediately after aortic crossclamping, cardioplegia was achieved by injection of a single dose of cold (4°C) crystalloid cardioplegic solution (Bretschneider solution, 30 mL/kg) into the aortic root. Additional topical cooling of the myocardium was performed by application of 500 mL 4°C cold saline solution. Myocardial temperature during CPB was monitored with a needle temperature probe placed into the ventricular septum (Temperature Sensing Catheter; Medtronic HemoTec, Inc, Englewood, Colo). At the end of CPB, anticoagulation was reversed by protamine. Mediastinal drains were placed, and the chest was closed.

Postoperative Care

The lungs of the pigs were mechanically ventilated until the end of the experiment (6 hours after sternal closure). Postoperative monitoring included continuous registration of heart rate and rhythm, mean arterial blood pressure, and left atrial pressure (LAP). Esophageal temperature and urinary output were also recorded. Target values for mean arterial blood pressure, LAP, and urinary output during the whole postoperative period were about 60 mm Hg, 5 to 9 mm Hg, and more than 1.5 mL/(kg $\cdot$ h), respectively. Inotropic support and diuretic treatment consisting of dopamine and furosemide, respectively, were adapted accordingly, as was volume substitution with a Ringer's lactate solution.

Tissue samples for reverse transcriptase (RT)–polymerase chain reaction (PCR) and Western blot were taken from the apex of the right ventricle before institution of CPB, before aortic crossclamping, and before opening of the aortic clamp. Additional sample was taken immediately post mortem for RT-PCR, Western blot, histologic study, and immunohistochemical study. Samples for RT-PCR and Western blot were rapidly excised, snap-frozen in liquid nitrogen, and then stored at -70°C until processing. For standard histologic and immunohistochemical studies, tissue specimens were fixed in 10% buffered formalin, embedded in paraffin, and cut in 4- to 6- μm thick serial sections.

TABLE 1. Primers used for polymerase chain reaction amplification

Product	Orientation	Gene bank accession No.	5'-3' Sequence
HSP-72	Sense Antisense	X68213	CCTTCACCGACACCGAGC TTGAAGTAGGCAGGCACGGT
Bcl-xL	Sense Antisense	AJ001203	GGTGGTTGACTTTCTCTCTAC CAAAAGTGTCAGCCGCC
Bak	Sense Antisense	AJ001204	TTTACGCCATGAGCAGG TAGCCGAAGCCCAGAAGAG
Fas	Sense Antisense	AJ001202	CTGTCAGCCATGCCCTC GCCATAACCACTGTAGG
β -actin	Sense Antisense	U07786	GGACTTCGAGCAGGAGATGG GCACCGTGTGGCGTAGAGG

Counting of Necrotic and Apoptotic Cells

Four serial sections from the myocardial probe were stained with hematoxylin and eosin. Morphologic criteria used to determine cell necrosis were cell swelling, disruption of cell membrane and cell architecture, lysis of the cell organelles and the nuclei, and hyper-eosinophilia.¹⁴ A cell was considered necrotic if at least one of these criteria was met. Necrotic cells were counted in 5 different fields of each slide by light microscopy at $\times 400$ magnification by two different investigators (M.Q., B.K.) blinded to the temperature group. Their counts differed by less than 10%. An average of 1000 cells were counted per slide; results are reported as the average of the percentages of necrotic cells counted by each investigator.

Detection of apoptotic cells was performed on replicates of the paraffin sections with terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling assay (ApoTag; Serologicals Corporation, Norcross, Ga) according to the manufacturer recommendations. For quantification of apoptosis, samples with labeled fluorescence staining were investigated with confocal laser scanning microscopy (Laser Scan Microscopy LSM 410 invert with Axiovert 135M; Carl Zeiss, Oberkochen, Germany). Apoptotic cells in 12 different fields were counted (an average of 1000 cells were counted per slide). Data were reported as the percentage of apoptotic cells, as averaged from the counts of both investigators.² Adjacent hematoxylin and eosin-stained sections were examined to assess typical morphologic findings of apoptosis (cell shrinkage, chromatin condensation and margination, and apoptotic bodies).¹⁴

Heat Shock Protein 72 Assay

HSP-72 immunohistochemical assay was performed on paraffin-embedded tissue sections with the IHC mouse kit (InnoGenex, Inc, San Ramon, Calif). Briefly, sections were pretreated with a solution of Peroxide Block to inhibit endogenous peroxidase activity and incubated with Power Block Reagent to block nonspecific protein binding. Subsequently, they were incubated with the monoclonal mouse anti-human HSP-72 antibody (1:200; Stress-Gen Biotechnologies Corp, Victoria, British Columbia, Canada) for 2 hours at room temperature. After rinsing with phosphate-buffered saline solution plus 0.1% polysorbate, the slides were incubated for 30 minutes with the secondary antibody. The slides were rinsed with phosphate-buffered saline solution and incubated

for 20 minutes in streptavidin-horseradish peroxidase conjugate. The color reaction was developed in aminoethyl carbazole substrate. Specimens were counterstained with Mayer hematoxylin. Appropriate positive and negative controls were used for primary antibody. Cellular localization of HSP-72 was analyzed with the Quantimet 600 image analyzer system (Leica Microsystems Nussloch GmbH, Nussloch, Germany).

Reverse Transcriptase-Polymerase Chain Reaction

Total RNA was extracted with the RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany). RNA (3 μ g) was reverse transcribed to complementary DNA with random hexamers. With pig-specific primers for HSP-72, Bcl-xL, Bak, Fas and β -actin (Table 1), complementary DNA products were coamplified by PCR. Thirty-five cycles for Bcl-xL, Bak, and Fas and 38 cycles for HSP-72 were performed after an initial denaturation at 95°C for 2 minutes: 30 seconds at 94°C, 30 seconds at 58°C (60°C for HSP-72), and 30 seconds at 72°C. PCR products were subjected to electrophoresis in 1.8% agarose gel, stained with ethidium bromide, and photographed. The predicted lengths of amplification products for the genes encoding HSP-72, Bcl-xL, Fas, Bak, and β -actin were 324, 289, 523, 372, and 233 base pairs, respectively. Results are presented as the ratio of the band intensities of the RT-PCR product to the corresponding messenger RNA (mRNA) encoding β -actin (Bio-Rad Multi-Analyst, Bio-Rad Laboratories Inc, Hercules, Calif).

Western Blot

Myocardial samples were homogenized in lysis buffer (1-mol/L tris[hydroxymethyl]aminomethane hydrochloride, pH 7.4, 0.5-mol/L ethylenediaminetetraacetic acid, 10% deoxycholate, and 10% nonidet P-40) containing the following protease and phosphatase inhibitors: pepstatin A (2 μ g/mL), leupeptin (5 μ g/mL), aprotinin (5 μ g/mL), and phenylmethylsulfonyl fluoride (1 mmol/L). Samples were incubated for 30 minutes at 4°C and centrifuged at 14,000g for 10 minutes at 4°C. Protein quantities were determined with a modified Bradford assay (Bio-Rad). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was conducted with the Laemmli buffer system on 12% polyacrylamide gels. Proteins (50 μ g for each lane) separated on gels were transferred to a polyvi-

TABLE 2. Esophageal and myocardial temperatures before, during, and after CPB in pigs operated on under moderate hypothermia or normothermia

Time point	Esophageal temperature			Myocardial temperature		
	28°C	37°C	<i>P</i> value	28°C	37°C	<i>P</i> value
Before CPB	36.0 ± 0.3	36.1 ± 0.2	>.2	35.3 ± 0.1	35.4 ± 0.2	>.2
Before aortic crossclamping	28.2 ± 0.3	35.9 ± 0.7	.005	28.1 ± 0.3	34.6 ± 0.4	<.001
Immediately after cardioplegia	27.9 ± 0.05	37.1 ± 0.3	.002	12.7 ± 0.6	13.9 ± 1.7	>.2
10 min after aortic crossclamping	27.9 ± 0.05	37.3 ± 0.4	.002	18.0 ± 1.7	18.8 ± 1.7	>.2
30 min after aortic crossclamping	27.9 ± 0.05	37.5 ± 0.5	.002	23.9 ± 1.1	27.7 ± 2.2	.081
60 min after aortic crossclamping	28.4 ± 1.4	35.8 ± 1.1	.012	28.4 ± 1.4	30.3 ± 1.5	>.2
Declamping of aorta	27.9 ± 0.05	37.4 ± 0.3	.002	29.2 ± 1.9	33.0 ± 1.5	.141
End of CPB	35.6 ± 0.3	36.9 ± 0.2	.029	34.9 ± 0.3	35.4 ± 0.8	>.2
6 h after CPB	35.9 ± 0.4	37.1 ± 0.6	.019	36.3 ± 0.8	38.2 ± 0.8	>.2

nylidene difluoride membrane (Millipore GmbH, Eschborn, Germany) with a semidry electroblotting apparatus. Binding of the primary and secondary antibodies was performed in phosphate-buffered saline solution (pH 7.5) containing 0.1% polysorbate 20 overnight at 4°C (primary antibodies) and for 1 hour at room temperature (secondary antibody). Primary antibodies used in immunoblotting were mouse anti-human HSP-72 antibody (1:1000 dilution, monoclonal; Stressgen Biotechnologies) and mouse anti-human β -actin antibody (1:2000 dilution, monoclonal; Sigma, St Louis, Mo). Polyclonal antimouse horseradish peroxidase-conjugated secondary antibodies (1:2000 dilution; DAKO Diagnostika GmbH, Hamburg Germany) were used to reveal the immunoreactive bands with the ECL chemiluminescence system according to the manufacturer instructions (Amersham Biosciences Europe GmbH, Freiburg, Germany). Signals for HSP-72 and β -actin produced bands of 70.5 kd and 43 kd, respectively. All protein signals for HSP-72 were normalized for β -actin signals that were developed on the same blot (NIH imaging 1.61b8. software; Bio-Rad).

Statistical Analysis

Results are expressed as mean $\pm$ SEM. Data were analyzed by analysis of variance with adjustment of *P* values by the Scheffé procedure for repeated comparisons. The *t* test was used for the pairwise comparison of mean values. Correlation analysis was performed with the Pearson correlation coefficient. Data were analyzed with SPSS statistical software package (SPSS Software GmbH, München, Germany).

Results

Esophageal and Myocardial Temperatures

Table 2 summarizes esophageal and myocardial temperatures during and after CPB. Esophageal temperatures during and after CPB were lower in pigs operated on under moderate hypothermia than in the other group. In contrast, intergroup differences in myocardial temperature during and after CPB were not observed or could have been due to chance, with the exception of that measured before cross-clamping of the aorta.

Hemodynamics

LAPs measured 1 and 4 hours after CPB tended to be lower in the hypothermia group than in the normothermia group (LAP 7.1 ± 0.7 mm Hg vs 9.3 ± 0.8 mm Hg and 7.3 ± 0.6 mm Hg vs 9.5 ± 0.9 mm Hg, respectively, *P* = .050). Heart rate, mean arterial pressure, and diuresis did not differ between the groups (data not shown).

Synthesis of Heat Shock Protein 72

HSP-72 expression was not detected in the myocardium at either the mRNA or the protein level before institution of CPB but was detected in all animals 6 hours after CPB. At that time, both HSP-72 gene expression and HSP-72 concentration were higher in pigs operated on under moderate hypothermia than in those operated on under normothermia (Figures 1 and 2). In the former group, HSP-72 expression was detected at both mRNA and protein levels as soon as 30 minutes after institution of CPB, before crossclamping of the aorta, and increased further during myocardial ischemia but no more after the operation. In contrast, in the normothermia group HSP-72 expression was not detected at either the mRNA or the protein level before CPB but was only detected at the end of the period of myocardial ischemia (90 minutes after institution of CPB) and did not increase further after the operation (Figures 1, A, and 2, A). Intramyocardial gene expression and concentrations of HSP-72 during and after CPB were higher in animals operated on under moderate hypothermia than in those operated on under normothermia (Figures 1, B, and 2, B). Expression of mRNA encoding β -actin was not affected by CPB.

Heat Shock Protein 72 Immunohistochemical Detection

In all animals, HSP-72 was present in the myocardium sampled 6 hours after CPB and was localized in cardiomyocytes, macrophages, and endothelial cells (Figure 3).

Figure 1. A, Expression of mRNA encoding HSP-72 in myocardium before CPB (1), before aortic crossclamping (2), before removal of aortic clamp (3), and 6 hours after CPB (4) in pigs operated on under moderate hypothermia (temperature 28°C, circles) and under normothermia (temperature 37°C, squares). Results are shown as ratio of mRNA encoding HSP-72 to that encoding β -actin detected by RT-PCR. Data points represent mean value; error bars represent SEM. Double asterisk indicates difference versus prebypass value in hypothermia group ($P = .042$). Asterisk indicates $P < .001$ between groups; dagger indicates $P = .002$; double dagger indicates $P = .007$. **B,** Effect of core temperature on myocardial expression of mRNA encoding HSP-72 detected by RT-PCR. Results are representative for 8 independent experiments in each group (moderate hypothermia 28°C, normothermia 37°C). M, Molecular weight markers; bp, base pairs.

Necrosis

The percentage of necrotic cells in the myocardium was lower in pigs operated on under hypothermia ($0.23\% \pm 0.13\%$) than in those operated on under normothermia ($2.57\% \pm 0.57\%$, $P = .002$).

Apoptosis

In all animals, mRNA encoding Fas, Bak, and Bcl-xL was not detectable in the heart before institution of CPB but was detectable 6 hours after CPB, without any intergroup differences (Table 3). At that time apoptotic bodies and apoptotic cells were also present in the myocardium of animals of both groups and averaged $0.82\% \pm 0.47\%$ in the animals operated on under hypothermia and $0.33\% \pm 0.24\%$ in those operated on under normothermia ($P > .2$).

Discussion

In this study, moderate hypothermia during CPB was associated with intramyocardial upward regulation of HSP-72 expression and with reduction of myocardial cell necrosis. This effect of moderate hypothermia on HSP-72 production is shown here for the first time in the setting of cardiac surgery but is supported by previous experimental studies. Indeed, in addition to such commonly accepted activators of the transcription factor heat shock factor 1 as heat, hypoxia, reactive oxygen species, and proinflammatory cytokines,⁹ a

few studies have reported that exposure to cold also induces gene expression of HSP-70.^{15,16} In an isolated rat heart model, hypothermic perfusion (31°C) preceding ischemia led to expression of mRNA encoding HSP-70.¹³ In vivo, however, recovery from hypothermia seems to be necessary for maximal stimulation of HSP-70 expression in response to heat shock factor 1.¹⁶

A main finding of this study was the presence of HSP-72 in the myocardium as early as 30 minutes after institution of hypothermic CPB, before institution of cold cardioplegic arrest. In contrast to that finding, HSP-72 was detectable only at the end of the period of myocardial ischemia in animals operated on under normothermia. This observation suggests that different kinds of stress induced HSP-72 during the course of cardiac surgery in both animal groups of this series. Indeed, 30 minutes after the beginning of CPB, both esophageal and myocardial temperature were (as expected) lower in the hypothermia than in the normothermia group. At that time, proinflammatory cytokine production is blunted in animals undergoing moderate hypothermic CPB but not in those operated on under normothermia, as we have recently shown.¹ This indicates the preponderant role of hypothermia in inducing HSP-72 during the early phase of hypothermic CPB. In contrast, proinflammatory mediators such as tumor necrosis factor (TNF) α , either released

Figure 2. A. Synthesis of HSP-72 measured by Western blot in myocardium before CPB (1), before aortic crossclamping (2), before removal of aortic clamp (3), and 6 hours after CPB (4) in pigs operated on under moderate hypothermia (temperature 28°C, circles) and under normothermia (temperature 37°C, squares). Band intensities for HSP-72 were normalized for band intensities of β -actin. Data points represent mean value; error bars represent SEM. Double asterisk indicates difference versus prebypass value in hypothermia group ($P = .068$). Asterisk indicates $P = .001$ between groups; dagger indicates $P = .085$; double dagger indicates $P = .058$. **B.** Effect of core temperature on myocardial expression of HSP-72 detected by Western blot. Results are representative for 8 independent experiments in each group (moderate hypothermia 28°C, normothermia 37°C). M, Molecular weight markers.

into the circulation or produced in the myocardium during myocardial ischemia,^{1,2} could be responsible for the later induction of HSP-72 in the normothermia group.¹⁷

Another main finding of this study was the significant reduction of cell death by necrosis but not by apoptosis related to moderately hypothermic CPB. HSP-70 is well known to play a key regulatory role in cell survival,¹⁸ protecting against cell necrosis.¹⁹ Our results showing that HSP-72-positive staining was present not only in parenchymatous cells but also in resident macrophages in the myocardium could indicate its inhibitory role on in situ macrophage- and TNF-mediated tissue injury.²⁰ Indeed, HSP-70 decreases TNF- α and interleukin 1 β production by binding nuclear factor κ B, preventing its translocation into the nucleus and thus reducing inflammatory response.²¹ In addition to this, HSP-70 prevents posttranslational release of TNF- α .²² This could also be a mechanism by which moderate hypothermia blunts TNF- α production during cardiac operations, as we have shown previously.^{1,2}

In contrast to their action against necrosis, the role of HSPs in the induction of apoptosis is controversial. Because they refold damaged repressors of the apoptotic pathway or prevent their degradation, HSPs have been implicated in the control of apoptosis.¹⁰ HSP-70 could also control apoptosis at the level of signal transduction of apoptosis-regulating proteins.²³ On the other hand, experimental studies have shown that HSPs are also inducers of apoptosis when non-lethal endotoxin stimulation occurs before a stress that

normally elicits HSP synthesis.²⁴ This points out the importance of quality and sequence of stresses imposed on the investigated organism in determining whether and how the cells will die.

In our series, although synthesis of HSP-72 was significantly higher in pigs operated on under moderate hypothermia than in the other animals, the levels of gene expression of apoptosis-regulatory proteins and amounts of apoptotic cells in the myocardium were similar in the two groups. This clearly indicates that in our model moderate hypothermia did not exert its protective effect by inhibiting apoptotic cell death. It is conceivable that different factors specific for each group of animals (hypothermic vs normothermic CPB) exerted apoptosis-inducing effects. Indeed, hypothermia itself is an inducer of apoptosis by inducing expression of the tumor suppressor protein p53, a temperature-sensitive protein.²⁵ On the other hand, proinflammatory cytokines released during normothermic CPB^{2,26} are inducers of apoptosis in various cell types.⁹ TNF- α upwardly regulates Fas expression and stimulates its type 1 receptor, inducing apoptotic events.²⁷ In addition, the anti-inflammatory cytokine interleukin 10, the circulating levels of which are increased in the early phase of hypothermic CPB as we have recently shown,¹ is an inducer of apoptosis in patients with sepsis and is thought to contribute to organ protection by enhancing the elimination of activated leukocytes.²⁸

It has recently been suggested that the presence of HSP-70 at the protein level is necessary for the inhibition of

Figure 3. Example of immunohistochemical study (original magnification 1000 $\times$) of right ventricular myocardium taken 6 hours after CPB in representative animal operated on under hypothermia showing the presence of HSP-72 (red staining) within myocardial cells (My, arrows), endothelial cells (E, arrows), and resident macrophages (M, arrows). C, Control probe taken before CPB.

TABLE 3. Effect of core temperature during CPB on intramyocardial gene expression of apoptosis regulatory proteins

	Hypothermia (28°C)	Normothermia (37°C)	P value
Bcl-xL	0.42 $\pm$ 0.05	0.28 $\pm$ 0.15	>.2
Bak	0.25 $\pm$ 0.1	0.12 $\pm$ 0.08	>.2
Fas	0.50 $\pm$ 0.12	0.67 $\pm$ 0.16	>.2

Results (mean $\pm$ SEM) are shown as the ratio of apoptosis-regulating protein mRNA to β -actin mRNA.

apoptosis and that the apoptosis-promoting protein Fas leads in turn to inhibition of the heat shock factor 1 and HSP-70 stress response.²⁹ However, there is usually a lack of linear correlation between the presence of HSPs and their protective role, indicating that a critical amount of HSP is necessary to elicit a protective effect.³⁰ Our results confirm this and show a relationship between overexpression of HSP-72 and reduction of cell death by necrosis.

In conclusion, in this study moderate hypothermic CPB, as compared with normothermic CPB, enhanced gene expression and synthesis of HSP-72 and reduced cell death by necrosis. Upward regulation of HSP-72 could be a mecha-

nism by which moderate hypothermia during cardiac operations protects against organ damage.

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