Heat shock proteins HSP70 and HSP27 in the cerebral spinal fluid of patients undergoing thoracic aneurysm repair correlate with the probability of postoperative paralysis

James G. Hecker · Hari Sundram · Shaomin Zou ·
Amy Praestgaard · Joseph E. Bavaria ·
Sindhu Ramchandren · Michael McGarvey

Received: 9 November 2007 / Revised: 18 March 2008 / Accepted: 19 March 2008 / Published online: 17 April 2008
© Cell Stress Society International 2008

Abstract An understanding of the time course and correlation with injury of heat shock proteins (HSPs) released during brain and/or spinal cord cellular stress (ischemia) is critical in understanding the role of the HSPs in cellular survival, and may provide a clinically useful biomarker of severe cellular stress. We have analyzed the levels of HSPs in the cerebrospinal fluid (CSF) from patients who are undergoing thoracic aneurysm repair. Blood and CSF samples were collected at regular intervals, and CSF was analyzed by enzyme-linked immunosorbent assay for HSP70 and HSP27. These results were correlated with intraoperative somatosensory-evoked potentials measure-

ments and postoperative paralysis. We find that the levels of these proteins in many patients are elevated and that the degree of elevation correlates with the risk of permanent paralysis. We hypothesize that sequential measurement intraoperatively of the levels of the heat shock proteins HSP70 and HSP27 in the CSF can predict those patients who are at greatest risk for paralysis during thoracic aneurysm surgery and will allow us to develop means of preventing or attenuating this severe and often fatal complication.

Keywords Aneurysm · Biomarkers · CSF · HSP70 · HSP27 · Ischemia · Neuroprotection · Paraplegia · Perfusion · Spinal cord · SSEP · TAAA · Thoracic

M.L. McGarvey and J.G. Hecker contributed equally to this work.

J. G. Hecker (✉) · H. Sundram · S. Zou
Department of Anesthesia, University of Pennsylvania,
305 Morgan, 3620 Hamilton Walk,
Philadelphia, PA 19104-6112, USA
e-mail: heckerj@uphs.upenn.edu

A. Praestgaard
Biostatistics Analysis Center, University of Pennsylvania,
Philadelphia, PA 19104, USA

M. McGarvey
Department of Neurology, University of Pennsylvania,
Philadelphia, PA 19104, USA

J. E. Bavaria
Cardiovascular Surgery, University of Pennsylvania,
Philadelphia, PA 19104, USA

S. Ramchandren
Department of Neurology, University of Michigan,
Ann Arbor, MI, USA

Abbreviations

TAA	thoracic aortic aneurysm
TAAA	thoraco-abdominal aortic aneurysm
DHCA	deep hypothermic circulatory arrest
LA/FA	left atrial femoral artery bypass
CABG	coronary artery bypass graft
HSP	heat shock protein
SSEP	somato sensory-evoked potentials
MEP	motor-evoked potential, EEG
TEVAR	thoracic aortic endovascular repair
MAP	mean arterial pressure
CNS	central nervous system
CSF	cerebral spinal fluid
ICP	intracerebral pressure
ASIA	American Spinal Injury Association
NIHSS	NIH Stroke Scale
RSE	residual squared error

Introduction

Despite clinical efforts to prevent spinal cord ischemia during thoracic aortic aneurysm (TAA) repair, paraplegia (paraparesis) remains a significant risk (Cina et al. 2004; Safi and Miller 1999). Any improvement in our ability to predict, detect, and intervene in the progression of ongoing spinal cord ischemia to prevent permanent damage would be a major advance and would be greatly aided by having a real-time, predictive marker for an elevated risk of paralysis. The heat shock proteins (HSPs) are members of a highly conserved family of molecular chaperones, some of which are rapidly induced by severe stress. The rapidly inducible form of HSP70 is normally near the lower limit of detection by enzyme-linked immunosorbent assay (ELISA; ~1 ng/ml) in the CSF of healthy humans (unpublished results from Dr. Hecker's IRB-approved human CSF collection study) and remains so under normal conditions, including, for example, exercise to exhaustion (Dalsgaard et al. 2004). The inducible members of the HSP70 and HSP27 families are associated with cellular protection and recovery after a near lethal stress and have also been used as markers for tissues or organs that have been exposed to a near-lethal stress (Beere 2005; Da Rocha et al. 2005); it is this feature that we will exploit in this study. Release, transcription, and translation of the inducible HSPs are among the fastest of intracellular severe stress responses. We propose that the heat shock proteins HSP70 and HSP27 are biomarkers that can detect *acute* spinal cord ischemia during thoraco-abdominal aortic aneurysm (TAAA) resection.

Methods

Surgical procedures

This study received IRB approval, and written informed consent was obtained from all study patients. All patients admitted to our institution for TAAA repairs from 2003 through 2006 who had lumbar CSF drains placed as part of the standard care were eligible for the study. Patients with significant preoperative neurologic deficits or acute preoperative neurologic events were excluded. Demographic data were collected preoperatively. Formal NIH Stroke Scale (NIHSS) and lower extremity American Spinal Injury Association (ASIA) scales were performed by a certified neurologist, who was not blinded to procedure or outcome, at baseline and 12, 24, and 48 h postoperatively and at discharge. Cases in which acute neurologic changes were detected were subjected to further NIHSS and ASIA exams during a detailed neurologic examination, along with appropriate neuroimaging to confirm the existence of spinal

or brain ischemia and exclude peripheral nerve or muscle injury. If an episode of spinal ischemia was detected intra- or postoperatively, our institution's standard protocol was followed to attempt to reverse ongoing ischemia (Cheung et al. 2002; Cheung et al. 2005; McGarvey et al. 2007). Briefly, this protocol involves pharmacologic elevation of the mean arterial pressure (MAP), increasing intravenous fluids, replacing the lumbar drain if it had been removed, and CSF drainage to 8–12 mmHg.

Clinical variables included age, race, gender, medical history, presentation status (emergent versus scheduled), whether deep hypothermic circulatory arrest (DHCA) was induced intraoperatively, whether intercostal vessels were re-implanted, presence of dissection or contained rupture, the repair type [cardiopulmonary bypass (CPB) with DHCA, left atrium/femoral artery bypass (LA/FA), thoracic endovascular aortic repair (TEVAR)], cross-clamp time, circulation arrest time, bypass time, maximal change in intraoperative MAP, and extent of repair. All patients received a narcotic-based anesthetic with inhaled isoflurane in oxygen. For open TAAA, circulation management consisted of either distal aortic partial left heart bypass with core cooling to 32°C (LA/FA bypass) or a deep hypothermic technique utilizing full cardiopulmonary bypass via the left chest with an open proximal anastomosis. Left atrium to femoral artery (LA/FA) bypass was used for aneurysms or dissections that did not involve the aortic root or aortic arch (type B or descending). If the arch vessels or aortic root were involved, either primarily or in a retrograde manner from the descending aorta, then deep hypothermic circulatory arrest (DHCA) with cardiopulmonary bypass was performed, with retrograde cerebral perfusion. For LA/FA management bypass, flow rates averaged 2.5 l/min, adjusted to achieve a target distal aortic perfusion pressure of at least 60 mmHg, while maintaining proximal aortic pressure of at least 90 mmHg. Repairs involving the distal arch repair required utilization of DHCA, systemic cooling on CPB until the electroencephalogram (EEG) reached electrical cerebral silence (12–18°C), then termination of CPB to perform the open proximal anastomosis with total body retrograde cerebral perfusion (300–500 cm/min at a central venous pressure of 12–15 mmHg) via an unsnared superior vena cava. After completion, arterial circulation was reinitiated via the proximal descending Dacron graft, the distal anastomosis was performed, and the patient was rewarmed. For combined distal arch and type II and III TAAA repairs, arterial circulation was reinitiated with both proximal and distal femoral artery perfusion. Lumbar CSF drains were used in both LA/FA bypass and hypothermic cases. The spinal cord central pressure is maintained at 10–12 mmHg with lumbar CSF drainage, in theory to maximize the perfusion pressure gradient between systemic circulation

and spinal cord. The lumbar CSF drain is usually clamped 24 h after surgery and removed after 48 h if there are neurologic deficits. Intercostal arteries were reimplanted in all patients when possible. Three patients in this cohort underwent descending thoracic aortic repair involving TEVAR without CPB involving general anesthesia, femoral artery cannulation, angiography, and aneurysm exclusion using an aortic stent graft.

CSF sample collection

Serial CSF samples were collected after informed consent as a part of our IRB-approved protocols at the following time points (when possible): (A) lumbar drain placement immediately after induction; (B) at the time of aortic cross-clamp in cases involving LA/FA bypass, or time of restart of CPB after DHCA, or aortic stent deployment in TEVAR procedures; (C) 1 h after B, (D) 2 h after B, (E) 12 h after B, and (F) 24 h after B. Further samples were collected at various time points if signs of paraparesis developed postoperatively. Samples were not collected if the ICP was not greater than 8 mmHg or the lumbar drain was not functional. Samples were immediately centrifuged, and the supernatants free of cell debris were stored frozen at -80°C .

We measured HSP70 and HSP27 in CSF samples after surgery was finished, and the postoperative outcomes of these patients were known. Patient results were divided into two subsets of patients: (1) patients with paraplegia ('permanent', or resolved with intervention, 'transient') and (2) patients with no signs or symptoms of paraplegia postoperatively ('normals'). Patients with intraoperative somato sensory-evoked potentials (SSEP) changes (with or without paraplegia) were noted and HSP results correlated with outcomes.

ELISA assay for HSP70 and HSP27

HSP70 and HSP27 were quantitatively measured with StressXpress HSP70 and HSP27 ELISA kits (EKS-700 and EKS-500, respectively Stressgen, Vancouver, BC, Canada). These kits use a mouse monoclonal antibody on pre-coated 96-well immunoassay plates. HSP70 and HSP27 were captured by immobilized antibodies by loading 100 μl CSF/well in duplicate on the plates, followed by incubation at 4°C overnight. After rinsing, captured HSP70 and HSP27 were probed by incubation at room temperature (RT) with secondary biotinylated rabbit polyclonal anti-mouse antibody (1:500 dilution). Unbound secondary antibodies were removed by rinsing. Biotinylated antibodies were incubated at RT with an avidin-horseradish peroxidase conjugate diluted 1:500. After rinsing, the assay was developed by incubation with tetramethylbenzidine substrate and intensity was measured in a microplate reader (Dynex Technology) at

450 nm. Standard curves were plotted on a log-log scale versus absorbance. Sequential sample results for HSP27 and HSP70 were tabulated and analyzed.

Statistical methods

All statistical analyses used the commercially available statistical packages (Statistical Applications Software Version 9.1, SAS Institute, Cary, NC, USA) and R (The R Project for Statistical Computing Version 2.6.0). The primary exposure variables were measurements of the human CSF proteins HSP27 and HSP70 taken at up to ten time points per patient during the surgical procedure or postoperatively. We also considered patient demographic characteristics and pre- and intra-surgical factors. Our primary outcome was the binary indicator of paralysis. To characterize the within-patient HSP27 and HSP70 values, we calculated the following summary statistics to serve as our primary exposure variables:

- The slope of the within-patient linear regression fit to HSP27 and HSP70 values observed over time
- The residual squared error (RSE) of the within-patient linear regression
- The maximum HSP27 and HSP70 values observed for each patient
- The ranges of the HSP27 and HSP70 values observed for each patient
- The within-patient change and percentage change from time point B to time point C (i.e., pre- and post-cross-clamp) HSP27 and HSP70 values
- The within-patient change and percentage change from the average pre- and post-cross-clamp HSP27 and HSP70 values

We characterized differences between binary paralysis outcome groups with respect to several demographic and surgical variables in addition to the HSP70 and HSP27 variables listed above. For discrete variables, we calculated the number of observations in each level/outcome group combination and tested for significant differences between groups with Fisher's exact tests. For continuous variables, we calculated medians and inter-quartile ranges and used the nonparametric Wilcoxon rank sum test to assess significant differences between outcome groups. All *p* values represent two-sided hypothesis tests.

We plotted mean and cumulative values of HSP27 and HSP70 at each time point to evaluate the relationship between changes in HSP measured in the CSF and postoperative paraplegia. We used boxplots to depict the relationship between the binary paralysis outcome and patient demographic variables and our calculated within-patient HSP70 and HSP27 values.

Multivariable logistic regression

We used a series of stepwise multivariable logistic regression to evaluate the effects of our primary exposure variables on the paralysis outcome. Each of the primary exposure HSP27 and HSP70 variables was considered in a separate model. We included demographic and surgical variables as potential confounders in each model; stepwise regression methods were employed to determine the best final model. The criterion for the stepwise selection of variables was $p < 0.25$. The *C*-Statistic was used to evaluate the goodness of fit of the models ($C = 0.80$).

Results

Forty-eight patients consented to participate in this study during the time period reported in this paper. Of the 48, surgery was canceled for one patient due to the presence of blood in the CSF at the time of lumbar drain placement. In 11 patients, a lumbar drain could not be placed for technical reasons, no CSF was collected, or surgery was cancelled for other reasons. We evaluated HSP70 and HSP27 in CSF samples after surgery was finished, and the postoperative outcomes of these patients were known. Patient results were divided into two mutually exclusive subsets of patients: (1) patients with paraplegia ('permanent', or resolved with intervention, 'transient') and (2) patients without signs or symptoms of paraplegia postoperatively ('normals').

Table 1 lists demographic, preoperative, and surgical statistics for the study population. Patients ranged in age from 40 to 80, with 20 men and 17 women. None of the demographic variables of age, race, and sex was significantly associated with the paralysis outcome. However, patient smoking history has a statistically significant effect on the paralysis outcome for our group of patients. Patients who developed postoperative paraplegia reported a median of 60 (inter-quartile range, 50 to 75) pack years, compared with only 7 (inter-quartile range, 0 to 45) pack years among patients who did not develop paraplegia ($p = 0.0011$). There were significantly more patients with chronic renal insufficiency (CRI) in the paralysis outcome group (40%, standard error = 26%) than in the normal outcome group (4.6%, standard error = 4.7%). Significantly fewer patients in the postoperative paralysis outcome group than in the normal outcome group underwent surgeries other than DHCA or LA/FA [0% versus 22.7% (standard error = 8.9%)]. This 'other' category includes three TEVAR (stent) patients and two patients who were repaired with open surgical procedures that did not require either DHCA or cardiac bypass.

Of the 37 non-consecutive thoraco-abdominal aneurysm patients we studied, 13 had large increases in HSP70 and HSP27 within 2 h of cross-clamping the aorta, and 12 more

had large increases within 48 h of cross-clamp. Of these 25, 13 had postoperative paraparesis of some degree. Twenty-two of the 25 patients who had significant HSP increases had either intraoperative SSEP or EEG changes consistent with brain or spinal cord ischemia, or neurological evidence of paraplegia or stroke postoperatively. Of the 12 patients who did not have significant HSP increases, only one had any evidence of paraplegia or central ischemia by intraoperative SSEP. Overall, an overwhelming majority of patients with either paraparesis and/or SSEP changes consistent with central ischemia had large increases in HSP70 and HSP27 at multiple time points in the perioperative period.

We plotted cumulative and average mean values of HSP70 and HSP27 at each time point after induction of anesthesia. The cumulative averages of HSP70 (shown in Fig. 1) and HSP27 levels from those patients who went on to develop paraplegia were larger than those of the patients who did not develop paraplegia. Mean values for HSP70 were also higher at each intra- or postoperative time point C through F for paraplegics versus normals, respectively (shown in Fig. 2). However, only HSP70 values at time points E and F were statistically significant in both Figs. 1 and 2 in these sample sizes. Graphs for HSP27 were similar (not shown).

We also plotted mean HSP70 and HSP27 levels to compare surgical technique (LAFA and DHCA) with changes in the heat shock proteins (HSP70 shown in Fig. 3, with similar results seen for HSP27). The percentage of patients who developed paraplegia was comparable in both open surgical repair subgroups (four out of ten DHCA patients, and ten out of 20 LAFA patients). However, the mean HSP70 levels at successive time points in patients with paraplegia after DHCA are considerably higher than for patients with paraplegia after LAFA, especially 12 and 24 h post cross-clamp (Fig. 3).

Figures 4 and 5 contain graphical and numerical comparisons of the paralysis and non-paralysis outcome groups with respect to each of the calculated variables listed above in Table 2. Figures 4 and 5 display side-by-side box-and-whisker plots of the independent variable distributions within the paralysis and non-paralysis groups for Hsp70 and Hsp27, respectively. In each box, the first quartile (Q1), median, and third quartile (Q3) are represented by the bottom of the box, thick line inside the box, and top of the box, respectively. "Whiskers" extend to the nearest data point within 1.5 times the inter-quartile range of the distribution. Individual points beyond the whiskers depict outliers. The p value resulting from a Kruskal–Wallis test of equal location parameters (i.e., medians) is given in Table 2. Small p values (< 0.05) indicate statistically significant differences between the two outcome groups. Finally, we explored the possibility that these effects are confounded

Table 1 Demographic and surgical statistics for study population

	Paraplegia=Yes	Paraplegia=No	<i>p</i> value
Population statistics			
Number of patients	15	22	
Age [median (25%, 75%)]	68 (61, 79)	66.5 (56, 77)	NS
Race [% (SE)]			
African American (% (SE))	13.3% (8.8%)	31.8% (9.9%)	NS
Caucasian	80% (10.3%)	63.6% (10.3%)	
Hispanic	6.7% (6.5%)	4.6% (4.7%)	
Sex [% (SE)]			
Male	60% (12.6%)	59.1% (10.5%)	NS
Female	40% (12.6%)	40.9% (10.5%)	
Preoperative statistics			
Number of patients	16	21	
Pack years [median (25%, 75%)]	60 (50, 75)	7 (0, 45)	0.0019
HTN [% (SE)]	93.3% (6.5%)	90.9% (6.1%)	NS
MI [% (SE)]	26.7% (11.4%)	27.3% (9.5%)	NS
COPD [% (SE)]	26.7% (11.4%)	27.3% (9.5%)	NS
DM [% (SE)]	0% (0.0%)	18.2% (8.2%)	NS
CRI [% (SE)]	40% (12.6%)	4.6% (4.5%)	0.0113
Afib [% (SE)]	6.7% (6.5%)	9.1% (6.1%)	NS
CAD [% (SE)]	0% (0.0%)	18.2% (9.2%)	NS
Stroke [% (SE)]	26.7% (11.4%)	18.2% (8.2%)	NS
CABG [% (SE)]	6.7% (6.5%)	0% (0.0%)	NS
Cholesterol [% (SE)]	6.7% (6.5%)	13.6% (7.3%)	NS
PUD [% (SE)]	0% (0.0%)	0% (0.0%)	NS
PVD [% (SE)]	13.3% (8.8%)	9.1% (6.1%)	NS
Surgical statistics			
Number of patients	16	21	
Cardiopulmonary bypass [% (SE)]	33.3% (12.2%)	27.3% (9.5%)	NS
Circ arrest [% (SE)]	26.7% (11.4%)	27.3% (9.5%)	NS
LAFA [% (SE)]	73.3% (11.4%)	50% (10.7%)	NS
Other [% (SE)]	0% (0.0%)	22.7% (8.9%)	0.0673
Bypass time [median (25%, 75%)]	131 (94, 176)	85.5 (0, 159)	0.0288
Circ arrest time [median (25%, 75%)]	0 (0, 14)	0 (0, 7)	NS
Cross-clamp time [median (25%, 75%)]	65 (59, 88)	65.5 (45, 79)	NS
Emergent presentations [% (SE)]	0% (0.0%)	18.2% (8.2%)	NS
Contained ruptures [% (SE)]	6.7% (6.5%)	9.1% (6.1%)	NS
Dissections [% (SE)]	20% (10.3%)	13.6% (7.3%)	NS
Stents [% (SE)]	0% (0.0%)	13.6% (7.4%)	NS
Active hypo [% (SE)]	26.7% (11.4%)	27.3% (9.5%)	NS
Reimplantation of intercostals [% (SE)]	46.7% (12.9%)	40.9% (10.5%)	NS

with basic patient demographic effects such as age, race, gender, and smoking history.

Patient smoking history has a statistically significant effect on the paralysis outcome for our group of patients. Patients with a longer smoking history are more likely to experience paralysis. As expected, increasing age and emergency status increase the risk for paraplegia, while race and gender did not impact outcome. The numerical summaries for the results depicted graphically in Figs. 4 and 5 are given in Table 2.

Table 3, which lists effects with *p* values <0.10, summarizes the detailed logistic regression results. The results are further evidence that, for our study group of 37 patients, HSP70 and HSP27 values observed during surgery

are indicative of paralysis. All significant effects are positive, which means that patients with higher values of the calculated quantities we considered were more likely to experience paralysis. More specifically, we conclude that patients with HSP70 and HSP27 measurements in CSF that were non-linear over time (i.e., higher RSE values), experienced more extremes (higher maximums and ranges), or had larger positive average changes from the pre- to post-cross-clamp time periods were more likely to experience paralysis than other patients. It is also interesting to note that patients with longer smoking histories were more likely to experience paralysis than others.

Patients with non-linear HSP27 and HSP70 changes over time, as seen in larger within-patient linear regression

Fig. 1 Average cumulative HSP70 measurements by paralysis outcome with 95% confidence bars

** Denotes statistical significance at 0.05.

Average Cumulative HSP70 and Number of Patients (N)

	Normal		Paraplegic	
	Avg Cum HSP70	N	Avg Cum HSP70	N
A	0.88	21	2.0	13
B	1.35	21	2.4	13
C	1.89	21	3.5	13
D	2.69	21	4.9	13
E	3.73	21	7.8	13
F	4.96	21	11.6	13

RSE values (Table 2), were more likely to experience paraplegia after surgery ($p=0.0010$ for HSP27 and $p=0.0005$ for HSP70). Larger maximum HSP27 measurements in CSF were observed in patients with postoperative paraplegia, with a median within-patient maximum HSP27 of 2.86 (inter-quartile range, 1.43 to 3.36) among paraplegics, compared with only 0.54 (inter-quartile range, 0.34 to 1.08) among non-paraplegics ($p=0.0006$). A similar comparison can be made for HSP70 within-patient maximum values ($p=0.0001$). Wider within-patient HSP ranges were observed among patients who developed paraplegia than among patients with a normal postoperative outcome [median range of 7.26 (inter-quartile range, 2.59 to 8.97) versus 1.60 (0.93, 2.27) for HSP70, $p<0.001$; median range of 2.22 (inter-quartile range, 1.09 to 3.12) versus 0.48 (0.19, 0.83) for HSP27, $p=0.0012$]. Patients with larger average HSP27 and HSP70 changes between the pre- and post-cross-clamp periods (time period B) were more likely to experience postoperative paraplegia. This was true both for average actual changes and for average percentage changes (please see Table 2 for detailed results, including p values).

Stepwise multivariable logistic regression results provide further evidence that, for our study group of 37 patients, within-patient patterns of pre- and intraoperative HSP27 and HSP70 measurements in the CSF are indicative of paralysis, even when we controlled for demographic and surgical variables (Table 3). More specifically, we verify first that, even after adjustment, patients with HSP measurements that were non-linear over time (i.e., higher RSE values) were more likely to experience postoperative paraplegia (HSP70 odds ratio=5.574, 95% confidence interval 1.538 to 20.193, $p=0.0089$). We also observe that patients with relatively extreme pre- and intraoperative HSP values were more likely to experience paralysis [maximum HSP70 odds ratio=1.870 (95% CI: 1.202 to 2.910), $p=0.0055$; within-patient HSP70 range odds ratio=1.907 (95% CI, 1.204 to 3.019), $p=0.0059$]. Finally, we conclude that, even after adjusting for demographic, preoperative, and intraoperative variables, larger positive average HSP27 changes from the pre- to post-cross-clamp time periods were indicative of paralysis [time point B to C percent change odds ratio=1.028 (95% CI, 0.999 to 1.057), $p=0.0617$; average AB to C + change odds ratio=3.815 (95% CI, 1.064 to 13.67), $p=0.0399$].

Fig. 2 Average HSP70 measurements by paralysis outcome with 95% confidence bars

** Denotes statistical significance at 0.05.

Average HSP70 and Number of Patients (N)

	Normal		Paraplegic	
	Average HSP70	N	Average HSP70	N
A	0.97	21	1.95	13
B	0.67	17	0.78	9
C	0.75	18	1.38	12
D	1.11	18	1.49	15
E	1.20	20	3.33	13
F	1.72	15	4.54	11

Discussion

As a corollary to our ongoing efforts to deliver transient neuroprotective genes to the central nervous system (CNS), we measured HSP27 and HSP70 expression as presumptive biomarkers for ischemic spinal cord injury in the CSF of patients who were undergoing TAA or TAAA repair. Previous work done by us and by others led us to hypothesize that HSP70 (Mariucci et al. 2007; Nowak and Jacewicz 1994; Westerheide and Morimoto 2006) and 27 levels (Latchman 2005; Patel et al. 2005; Wagstaff et al. 1999) would be found at significant levels in the CSF in the presence of CNS ischemia intraoperatively, and that the quantitative HSP70/27 level would be predictive of the severity of ongoing ischemia and the potential for paralysis. Knowledge of elevated HSP70 and HSP27 levels in the CSF would allow the anesthesiologist, neurologist, and surgeon the opportunity to alter surgical procedures or, if elevated preoperatively, to cancel or modify a surgical resection. We have confirmed this hypothesis.

The highly conserved heat shock proteins have multiple roles in vivo. HSP70 mediates cell protection and homeostasis, and recent evidence suggests that HSP70 is important in the regulation of apoptosis and inflammatory

responses as well (Beere 2004). Numerous studies have used HSP70 immunohistochemistry as a marker for injury, for example, after cardiac surgery (Becker et al. 2007; Dybahl et al. 2004) or in the CNS (Chen et al. 1998; Lindsberg et al. 1996; Mariucci et al. 2007; Nowak and Jacewicz 1994). Transcription and translation of these proteins increases dramatically in response to hypoxia or ischemia (Li et al. 2004; Nowak and Jacewicz 1994). This serves as an endogenous mediator of intracellular protection, not just in the CNS but in all tissues. Regner et al. (Da Rocha et al. 2005) showed that elevated serum HSP 70 levels, for example, predicted a poor outcome after severe traumatic brain injury in patients. Hart et al. (Carmel et al. 2004; Cizkova et al. 2004) showed a robust induction of HSPs with 6 and 12 min of spinal cord ischemia in a microarray analysis. Contreras (Contreras et al. 2005) demonstrated spinal cord protection using immediate ischemic pre-conditioning guided by SSEP measurements but did not measure HSPs as a part of this study. Robinson et al. (2005) showed an increase in HSP70 in response to ischemia in motoneurons, which reduced damage. They also showed that in vitro application of exogenous HSP70 to neuronal cells with conferred protection and improved long term motor neuron survival. We are most interested in

Fig. 3 Average HSP70 measurements by type of surgery with 95% confidence bars

Fig. 4 Within-patient HSP70 summaries by paralysis outcome

Fig. 5 Within-patient HSP27 summaries by paralysis outcome

the CNS, where multiple groups have also shown that the inducible heat shock proteins, in particular the 70-kDA HSP70, confer neuroprotection in the brain from injury (Kelly and Yenari 2002; Lee et al. 2006; Rajdev et al. 2000; Tsuchiya et al. 2003; van der Weerd et al. 2005). These multiple roles for HSP70 and HSP27 led us to investigate in

this study whether quantitative measurements of HSP70 and HSP27 can be used to predict ischemic injury.

We assume that CSF HSP levels reflect spinal cord ischemia due to the type and location of surgery. However, as HSPs are induced in any tissue that is exposed to a near-lethal stress, CSF HSPs could also be due to brain ischemia

Table 2 Within-patient HSP statistics

	Paraplegia = 1	Paraplegia = 0	<i>p</i> value
	Median (25%, 75%)		
HSP70			
Number of patients	15	22	
Linear regression sope	0.52 (0.13, 1.04)	0.20 (0.03, 0.36)	0.0946
Linear regression residual squared error	1.92 (1.11, 2.71)	0.51 (0.42, 0.79)	0.0005
Maximum	8.16 (3.01, 9.19)	1.80 (1.13, 3.04)	0.0001
Range	7.26 (2.59, 8.97)	1.60 (0.93, 2.27)	< 0.0001
Change from B to C	0.05 (−0.08, 0.85)	0.12 (−0.31, 0.25)	NS
Percent change from B to C	10.21 (−20.26, 51.15)	20.59 (−37.63, 46.83)	NS
Average AB to C + change	2.13 (0.53, 3.45)	0.39 (−0.16, 0.77)	0.0173
Average AB to C + percent changed	263.08 (61.00, 306.75)	70.35 (−26.30, 127.64)	0.0424
HSP27			
Number of patients	13	22	
Linear regression slope	0.19 (0.07, 0.39)	0.06 (0.02, 0.10)	0.0187
Linear regression residual squared error	0.61 (0.37, 1.04)	0.17 (0.09, 0.35)	0.0010
Maximum	2.86 (1.43, 3.36)	0.54 (0.34, 1.08)	0.0006
Range	2.22 (1.09, 3.12)	0.48 (0.19, 0.83)	0.0012
Change from B to C	0.14 (0.04, 1.01)	0.00 (−0.30, 0.02)	0.0169
Percent change from B to C	50.09 (11.41, 60.85)	−6.48 (−30.60, 30.97)	0.0290
Average AB to C + change	1.01 (0.27, 1.39)	0.12 (0.00, 0.20)	0.0072
Average AB to C + percent changed	172.48 (47.41, 382.35)	84.06 (−4.30, 335.374)	NS

Table 3 Multivariable logistic regression results

Primary exposure variable:	Odds ratio (95% confidence interval)	<i>p</i> value
HSP70		
Linear regression slope	2.852 (0.523, 15.546)	NS
Linear regression residual squared error	5.574 (1.538, 20.193)	0.0089
Maximum	1.870 (1.202, 2.910)	0.0055
Range	1.907 (1.204, 3.019)	0.0059
Change from B to C	2.455 (0.515, 11.691)	NS
Percent change from B to C	1.001 (0.986, 1.016)	NS
Average AB to C + change	1.149 (0.843, 1.567)	NS
Average AB to C + percent changed	1.002 (0.999, 1.006)	NS
HSP27		
Linear regression slope	675.293 (3.294, >999.999) ^a	0.0164
Linear regression residual squared error	>999.999 (3.740, >999.999) ^a	0.0226
Maximum	6.132 (1.470, 25.568)	0.0128
Range	6.038 (1.451, 25.124)	0.0134
Change from B to C	>999.999 (0.063, >999.999) ^a	NS
Percent change from B to C	1.028 (0.999, 1.057)	0.0617
Average AB to C + change	3.815 (1.064, 13.67)	0.0399
Average AB to C + percent changed	1.001 (0.999, 1.002)	NS

^a Large sample approximation to confidence interval. The >999 value for the odds ratio means that the estimate is unstable, mostly due to the small sample size. These types of models are iterative, and in order to settle on a “stable” point estimate, need to converge to a single value.

or even due to increased systemically circulating levels if the blood brain barrier is not intact. For example, in only one patient was the initial HSP70 level significantly greater than 2.0 at the first time point. This patient had had a carotid endarterectomy (CEA) the previous day, with a normal post-CEA neurological exam. By measuring HSP70 and HSP27 in the CSF at multiple time points, we have assured ourselves that a significant increase in HSP is not an isolated time point. Rapid measurement of these HSP levels would allow spinal cord preservation techniques to be utilized, some of which are done routinely already, others not. These include draining CSF to maximize perfusion gradient, increasing MAP, cooling the epidural space, reimplantation of additional intercostal arteries, and advising the surgeons to consider alternative operative procedures.

Our data support the hypothesis that there is a significant expression of HSP70 and HSP27 when the spinal cord is exposed to ischemia. HSP values appeared to be more sensitive than SSEP (data not shown here) for predicting postoperative paraplegia, as a greater percentage of patients with increases in HSPs and persistently elevated levels of HSPs end up paraplegic than those with SSEP changes. The combination of SSEP changes and HSP70 levels might be even more useful in combination. In the postoperative period, patients are not monitored by SSEP, but HSP levels can be measured until removal of the lumbar drain. We were somewhat limited by the number of samples that were available for analysis. More frequent sample collection during the first 6 to 12 h after cross-clamp might allow a critical threshold for ‘impending, irreversible spinal cord ischemia’ to be determined. Some combination of the magnitude of HSP elevation (RSE<slope, range) and

duration of increased HSP might well be highly predictive of impending irreversible ischemia.

We have noticed several other intriguing observations that will require additional patients to fully resolve. Although there are three surgical approaches, thoracic endovascular aortic repair (TEVAR), LA/FA partial bypass, and DHCA with full cardiopulmonary bypass (CBP), only LAFA and DHCA are open procedures. The percentage of patients who developed paraplegia was comparable in both groups of open repairs (four out of ten DHCA patients, and ten out of 20 LAFA patients). However, the mean HSP70 levels at successive time points in patients with plegia after DHCA are considerably higher than for patients with plegia after LAFA, especially 12 and 24 h post-cross-clamp. This trend (which was not statistically significant) was seen both in patients with paraplegia and in patients who had no postoperative neurological complications. It is unclear whether DHCA has a greater degree of ischemic injury than LAFA, whether this is brain or spinal cord injury, and whether these patients are thus afforded protection from even greater injury by the induction of hypothermia. It is possible that these patients are suffering a sub-clinical neurocognitive injury that has been associated with full cardiopulmonary bypass. LA/FA patients who do not undergo bypass and are kept warmer (typically 32°C during bypass) during the operation, not only had paralysis at lower mean HSP 70 levels but also appeared to be at greater risk of permanent neurological injury. Two out of the four patients with neurological compromise after DHCA recovered fully, whereas only two of the ten patients in the LAFA group with similar complications recovered even partial function with intervention. The HSP70 level elevation noted in DHCA patients may represent a significantly

higher level of ischemia that results from this operative approach due to a reliance on retrograde blood flow plus cardiopulmonary bypass to provide cerebral perfusion, or the elevation in HSP 70 may reflect increased expression of HSP70 systemically in DHCA, as hypothermia and hyperthermia are both known to induce HSP70. DHCA patients may reflect more extensive or complicated repairs and may be exposed to a double or triple insult: Severe hypothermia, complete cessation of CNS blood flow, and simultaneous cardiovascular bypass [although the interaction of cold, reperfusion, and coronary artery bypass graft (CABG) are poorly understood at best]. Further studies will be necessary to determine the neuroprotective effects of DHCA and the potentially lower risks associated with TEVAR.

The analysis results we have presented in this study clearly demonstrate a relationship between within-patient HSP27 and HSP70 values and paralysis. We do note that this study is observational in nature and that more data must be collected to make general conclusions. In general, we see that patients with non-linear, highly variable HSP values are more likely to experience paralysis as an outcome. This can provide the physician with an early warning for intervention. The next phase of analysis will focus on finding an actionable threshold for HSP values so that interventions during surgery can take place. We are continuing to collect data on additional patients both to validate these initial results and to further explore relationships between measurements taken during surgery and the risk (predictability) of paralysis.

Acknowledgments We would like to express our gratitude for the valuable expertise of, skillful intraoperative management by, and discussions with our cardiac anesthesia colleagues, Drs. Augoustides, Cheung, Floyd, Hanson, Horak, Levy, Savino, and Weiss. We also gratefully acknowledge the cooperation of the Aortic Surgery Group of Drs. Acker, Bavaria, Pochettino, Szeto, and Woo. We also thank Dr. Andrew Ochroch for preliminary statistical analysis.

References

- Becker S, Wendel H, Vonthein R, Rennekampff H, Aebert H (2007) Inducible HSP70 levels in thoracic wound fluid indicate myocardial damage after open heart surgery. *J Cardiovasc Surg* 48:233–237
- Beere HM (2004) “The stress of dying”: the role of heat shock proteins in the regulation of apoptosis. *J Cell Sci* 117:2641–2651
- Beere HM (2005) Death versus survival: functional interaction between the apoptotic and stress-inducible heat shock protein pathways. *J Clin Invest* 115:2633–2639
- Carmel JB, Kakinohana O, Mestral R, Young W, Marsala M, Hart RP (2004) Mediators of ischemic preconditioning identified by microarray analysis of rat spinal cord. *Exp Neurol* 185:81–96
- Chen M, Clark R, Kochanek P, Chen J, Schilding J, Stetler R, Simon RP, Graham S (1998) 72-kDa heat shock protein and mRNA expression after controlled cortical impact injury with hypoxemia in rats. *J Neurotrauma* 15:171–181
- Cheung AT, Weiss SJ, McGarvey ML, Stecker MM, Hogan MS, Escherich A, Bavaria JE (2002) Interventions for reversing delayed-onset postoperative paraplegia after thoracic aortic reconstruction. *Ann Thorac Surg* 74:413–419
- Cheung AT, Pochettino A, McGarvey ML, Appoo JJ, Fairman RM, Carpenter JP, Moser WG, Woo EY, Bavaria JE (2005) Strategies to manage paraplegia risk after endovascular stent repair of descending thoracic aortic aneurysms. *Ann Thorac Surg* 80:1280–1289
- Cina CS, Abouzahr L, Arena GO, Lagana A, Devereaux PJ, Farrokhfar F (2004) Cerebrospinal fluid drainage to prevent paraplegia during thoracic and thoracoabdominal aortic aneurysm surgery: a systematic review and meta-analysis. *J Vasc Surg* 40:36–44
- Cizkova D, Carmel JB, Yamamoto K, Kakinohana O, Sun D, Hart RP, Marsala M (2004) Characterization of spinal HSP72 induction and development of ischemic tolerance after spinal ischemia in rats. *Exp Neurol* 185:97–108
- Contreras IS, Moreira LF, Ballester G, de Monaco BA, Lancellotti CL, Dias AR, Oliveira SA (2005) Immediate ischemic preconditioning based on somatosensory evoked potentials seems to prevent spinal cord injury following descending thoracic aorta cross-clamping. *Eur J Cardio-thorac Surg* 28:274–279
- Da Rocha AB, Zanoni C, De Freitas GR, Andre C, Himelfarb S, Schneider RF, Grivicich I, Borges L, Schwartzmann G, Kaufmann M, Regner A (2005) Serum Hsp70 as an early predictor of fatal outcome after severe traumatic brain injury in males. *J Neurotrauma* 22:966–977
- Dalsgaard MK, Ott P, Dela F, Juul A, Pedersen BK, Warberg J, Fahrenkrug J, Secher NH (2004) The CSF and arterial to internal jugular venous hormonal differences during exercise in humans. *Exp Physiol* 89:271–277
- Dybahl B, Wahba A, Haaverstad R, Kirkeby-Garstad I, Kierulf P, Espevik T, Sundan A (2004) On-pump versus off-pump coronary artery bypass grafting: more heat-shock protein 70 is released after on-pump surgery. *Eur J Cardio-thorac Surg* 25:985–982
- Kelly S, Yenari M (2002) Neuroprotection: heat shock proteins. *Curr Med Res Opin* 18:55–60
- Latchman D (2005) HSP27 and cell survival in neurones. *Int J Hypertherm* 21:393–402
- Lee W, Wen H, Chang C, Chen M, Lin M (2006) Heat shock protein 72 overexpression protects against hyperthermia, circulatory shock, and cerebral ischemia during heat stroke. *J Appl Physiol* 100:2073–2082
- Li Z, Zhao X, Wei Y (2004) Regulation of apoptotic signal transduction pathways by the heat shock proteins. *Sci China C Life Sci* 47:107–114
- Lindsberg P, Frerichs K, Siren A, Hallenbeck J, Nowak T (1996) Heat-shock-protein and C-fos expression in focal microvascular brain damage. *J Cereb Blood Flow Metab* 16:82–91
- Mariucci G, Tantucci M, Giuditta A, Ambrosini M (2007) Permanent brain ischemia induces marked increments in hsp72 expression and local protein synthesis in synapses of the ischemic hemisphere. *Neurosci Lett* 415:77–80
- McGarvey ML, Mullen MT, Woo EY, Bavaria JE, Augoustides YG, Messe SR, Cheung AT (2007) The treatment of spinal cord ischemia following thoracic endovascular aortic repair. *Neurocritical Care* 6:35–39
- Nowak TS Jr., Jacewicz M (1994) The heat shock/stress response in focal cerebral ischemia. *Brain Pathol* 4:67–76
- Patel Y, Payne Smith M, de Belleruche J, Latchman D (2005) Hsp27 and Hsp70 administered in combination have a potent protective effect against FALS-associated SOD1-mutant-induced cell death in mammalian neuronal cells. *Brain Res Mol Brain Res* 134:256–274

- Rajdev S, Hara K, Kokubo Y, Mestril R, Dillmann W, Weinstein PR, Sharp F (2000) Mice overexpressing rat heat shock protein 70 are protected against cerebral infarction. *Ann Neurol* 47:782–791
- Robinson MB, Tidwell JL, Gould T, Taylor AR, Newbern J, Graves J, Tytell M, Milligan CE (2005) Extracellular heat shock protein 70: a critical component for motoneuron survival. *J Neurosci* 25: 9735–9745
- Safi HJ, Miller CC III (1999) Spinal cord protection in descending thoracic and thoracoabdominal aortic repair. *Ann Thorac Surg* 67:1937–1939
- Tsuchiya D, Hong S, Matsumori Y, Kayama T, Swanson R, Dillmann W, Liu J, Panter S, Weinstein P (2003) Overexpression of rat heat shock protein 70 reduces neuronal injury after transient focal ischemia, transient global ischemia, or kainic acid-induced seizures. *Neurosurgery* 53:1179–1188
- van der Weerd L, Lythgoe M, Badin R, Valentim L, Akbar M, de Belleruche J, Latchman D, Gadian D (2005) Neuroprotective effects of HSP70 overexpression after cerebral ischaemia—an MRI study. *Exp Neurol* 195:257–266
- Wagstaff M, Collaco-Moraes Y, Smith J, de Belleruche J, Coffin R, Latchman D (1999) Protection of neuronal cells from apoptosis by Hsp27 delivered with a herpes simplex virus-based vector. *J Biol Chem* 274:5061–5069
- Westerheide SD, Morimoto RI (2006) Heat shock response modulators as therapeutic tools for diseases of protein conformation. *J Biol Chem* 280:33097–33100