Heat shock protein 27 is increased in cyanotic tetralogy of Fallot myocardium and is associated with improved cardiac output and contraction

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Abstract Tetralogy of Fallot (TOF) is a congenital heart condition in which the right ventricle is exposed to cyanosis and pressure overload. Patients have an increased risk of right ventricle dysfunction following corrective surgery. Whether the cyanotic myocardium is less tolerant of injury compared to non-cyanotic is unclear. Heat shock proteins (HSPs) protect against cellular stresses. The aim of this study was to examine HSP 27 expression in the right ventricle resected from TOF patients and determine its relationship with right ventricle function and clinical outcome. Ten cyanotic and ten non-cyanotic patients were studied. Western blotting was used to quantify HSP 27 in resected myocardium at (1) baseline (first 15 min of aortic cross clamp and closest representation of pre-operative status) and (2) after 15 min during ischemia until surgery was complete. The cyanotic group had significantly increased haematocrit, lower O₂ saturation, thicker interventricular septal wall thickness and released more troponin-I on post-operative day 1 ($p<0.05$). HSP 27 expression was significantly increased in the <15 min cyanotic compared to the <15 min non-cyanotic group ($p=0.03$). In the cyanotic group, baseline HSP 27 expression also significantly correlated with oxygen extraction ratio ($p=0.028$), post-operative basal septal velocity ($p=0.036$) and mixed venous oxygen saturation ($p=0.02$), markers of improved cardiac output/contraction. Increased HSP 27 expression and associated improved right ventricle function and systemic perfusion supports a cardio-protective effect of HSP 27 in cyanotic TOF.

Keywords Tetralogy of Fallot · Heat shock protein 27 · Right ventricle · Myocardium · Cyanotic · Non-cyanotic

Introduction

Tetralogy of Fallot (TOF) is the most common cyanotic congenital heart disease with an incidence of four per 10,000 live births. Complete repair can be performed with a low mortality risk (<2 %) (Starr 2010). Early outcome can be determined by right ventricle function in the immediate post-operative period. In the 20 years after repair, 10 % to 15 % of patients will require further operation on their right ventricle outflow tract, most for limited exercise capacity, ventricular dysrhythmias or more severe symptoms of heart failure (Nollert et al. 1997). TOF is an example of a congenital heart condition in which the right ventricle is exposed to cyanosis and pressure overload. Patients have an increased risk of right ventricle dysfunction following corrective surgery. The causes include residual haemodynamic load from pulmonary stenosis or regurgitation, conduction abnormalities, residual right ventricle outflow tract obstruction and direct surgical trauma. How adequate the myocardium is protected during surgical repair has been an area of concern. Pre-operatively, the ventricle is exposed to increased systemic pressure. Furthermore, cardiopulmonary bypass exposes the myocardium to ischemic stress/hypoxia (during bypass) and potential reperfusion injury when oxygen is restored at super-physiological levels. Whether the chronically hypoxic (cyanotic) myocardium is more or less tolerant of injury compared to normoxic (non-cyanotic) patients is unclear. Free radical injury has been reported during surgery as well as a non-homogeneous distribution of cardioplegia in what is a hypertrophied right ventricle mass (Imura et al. 2001).

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The heart adapts to an adverse physiological environment by altering gene expression (Ghorbel et al. 2010). Cyanotic TOF is associated with higher mRNA expression of genes associated with apoptosis and remodeling and reduced expression of genes associated with myocardium contractility and function as well as genes that protect against re-oxygenation injury compared with non-cyanotic TOF (Willis and Patterson 2010), and this could limit the ability of the right ventricle (RV) to protect itself against ischaemic injury. An increase in ventricular mass will increase the susceptibility to ischaemic injury further. In TOF, very young patients are subjected to complex open heart surgery along with cardiopulmonary bypass. The ability of this “immature” myocardium to protect itself against ischemic (and reperfusion) injury during aortic cross clamping will determine post-operative outcome.

Heat shock proteins (HSPs) are a family of proteins that can be constitutively expressed in cells or induced by a variety of stress stimuli such as hypoxia, ischemia and pressure overloading (Willis and Patterson 2010). The best described role for HSPs is maintaining the correct folding of proteins (Willis and Patterson 2010). Although studied in other fields of medicine, the role of HSPs before, and following paediatric cardiac surgery, is not understood. However, it is becoming clear from adult human and animal studies that HSPs may have an important role in protecting the damaged heart, which accumulates misfolded proteins (Schmitt et al. 2002; Taggart et al. 1997; Jayakumar et al. 2000; Hollander et al. 2004; Yet et al. 2001; Aloy et al. 2008; Franklin et al. 2005; Efthymiou et al. 2004 and Knowlton et al. 1998).

Only a few of the smaller HSPs, including HSP 27, are increased in cells subjected to stress (Efthymiou et al. 2004). HSP 27 is involved in protein folding and in the apoptotic/programmed cell death pathway. Only a small number of studies on HSP 27 have been performed on the heart. These have been restricted mainly to animal studies and a few to adult disease. They do suggest that HSP 27 is protective for the heart (VanderHeide 2002).

The role of HSP 27 in the infant heart in TOF or during cardiopulmonary bypass, which itself is an ischemic–reperfusion insult, is not known. Thus, the aim of this study was to determine the expression of HSP 27 protein in myocardium resected from both cyanotic and non-cyanotic TOF patients during surgical repair and to determine its relationship to right ventricle function and clinical outcome.

Materials and methods

Twenty patients with TOF who underwent surgical repair at the Royal Hospital for Sick Children, Glasgow were recruited for the study. Patients were divided into a cyanotic group ($n=10$) and non-cyanotic group ($n=10$) based on pre-

operative saturations at rest $<90\%$ and $>90\%$ on room air, respectively. The ages of the two groups are shown in Table 1. A separate control group of age-matched children ($n=15$) presenting with an innocent murmur (median age, 27.1; interquartile range, 4.0–47.2 months versus TOF patients; $p=0.62$) was recruited to obtain normal reference values for tissue Doppler myocardial velocities. The study was approved by the Glasgow West Research Ethics Committee. Parental consent was obtained for all patients.

Perioperative tissue Doppler echocardiography

Tissue Doppler echocardiography was performed under general anaesthesia as described previously (Peng et al. 2011) before skin incision and repeated on post-operative day 1 in the intensive care unit (ICU) and then 1 week later. Pulsed wave tissue Doppler velocity measurements at the tricuspid annulus, basal septum and mitral annulus were obtained from an apical four-chamber view to quantify systolic myocardial velocity. Three or more loops were stored for subsequent offline analysis. The presence of restrictive right ventricle physiology was defined as antegrade flow across the pulmonary artery coincident with atrial systole in all respiratory cycles [parasternal short axis right ventricle outflow tract view] (Cullen et al. 1995).

Surgical repair and myocardial protection

Standard repair was performed as described previously (Peng et al. 2011) by ventricular septal defect (VSD) closure and resection of right ventricle infundibular muscle via transtricuspid and transpulmonary routes using moderate hypothermia and blood cardioplegia. Prior to cardiopulmonary bypass, all patients received oxygen supplementation and fractional inspired oxygen was adjusted to maintain adequate oxygenation whilst avoiding hyperoxia (median P_aO_2 , 8.0 kPa in the cyanotic group versus 12.1 kPa in the non-cyanotic group; $p=0.2$). Epicardial echo was routinely performed to exclude any residual lesion at the end of the procedure. Direct right ventricle and left ventricle pressures were measured intra-operatively post-repair.

Western blot analysis

All myocardia resected from the right ventricular outflow tract, as part of the repair, were collected for protein analysis. Muscle was snap frozen immediately following resection. Pieces of muscle were grouped according to the ischemic time point at collection. The beginning of ischemic time was defined as the time when aortic cross clamping

Table 1 Peri-operative characteristics between cyanotic and non-cyanotic group

	Cyanotic group (<i>n</i> =10)	Non-cyanotic group (<i>n</i> =10)	<i>P</i> value	95 % CI of median difference
Pre-operative factors				
Age (months)	22.1 (11.8–24.3)	16.4 (6.8–23.4)	0.36	(−3.8, 16.3)
Gender (<i>n</i> , %)	6 males (60 %)	5 males (50 %)	1.00	–
Weight (kg)	9.2 (8.6–12.2)	10.5 (7.9–11.0)	0.94	(−2.2, 3.2)
Haematocrit level (%)	47.0 (40.8–50.0)	38.1 (37.0–40.9)	0.01	(1.9, 11.3)
O ₂ saturation (%)	76 (68–86)	93 (91–97)	0.0002	(−26, −10)
BTS (<i>n</i> , %)	6, 60 %	2, 20 %	0.17	–
RV wall thickness (cm)	0.70 (0.58–0.83)	0.65 (0.57–0.82)	0.91	(−0.13, 0.16)
IVS wall thickness (cm)	0.85 (0.70–0.94)	0.66 (0.54–0.75)	0.01	(0.08, 0.34)
Sm–tricuspid annulus (cm/s)	10.01 (7.28–11.55)	7.85 (7.49–10.13)	0.31	(−1.12, 3.64)
Sm–basal septum (cm/s)	4.93 (4.18–6.03)	4.69 (3.73–6.10)	0.53	(−1.52, 0.82)
Sm–mitral annulus (cm/s)	4.90 (3.33–6.23)	4.83 (3.95–5.63)	0.96	(−1.47, 1.72)
Intra-operative factors				
Cross clamp time (min)	100.0 (75.0–130.0)	67.5 (61.8–76.0)	0.004	(−58.0, −8.0)
Bypass time (min)	218.5 (180.8–310.0)	114.5 (98.8–130.3)	0.003	(63.0, 182.0)
Transannular patch use (<i>n</i> , %)	6.60 %	4. 40 %	0.67	–
Subvalvar patch (<i>n</i> , %)	5.50 %	2.20 %	0.35	–
RV to systemic pressure ratio HSP 27 values	0.79 (0.69–1.11)	0.72 (0.62–0.88)	0.41	(−0.12, 0.35)
Median HSP 27 in baseline first 15-min period (R.O.D.)	0.79(0.68–1.6)	0.71 (0.5–0.85)	0.03	
Median HSP 27 after the first 15-min period (R.O.D.)	0.83 (0.7–1.0)	0.72 (0.53–0.88)	0.48	
Median HSP 27i from XC period (R.O.D.) (<15+>15 min)	0.82 (0.68–1.4)	0.72 (0.5–0.85)	0.01	
Post-operative factors (day 1)				
Troponin-I (ng/mL)	16.5 (11.7–51.0)	11.1 (7.6–13.5)	0.04	(0.1, 37.6)
Inotrope score	14.0 (9.5–16.3)	6.5 (0.8–14.9)	0.05	(0.0, 13.0)
Haemoglobin (g/dL)	12.2 (11.4–14.0)	11.5 (10.4–13.6)	0.41	(−1.2, 2.1)
SvO ₂ (%)	65.4 (55.5–71.3)	66.5 (61.8–68.3)	0.91	(−9.0, 7.0)
Oxygen extraction ratio	0.33 (0.28–0.42)	0.32 (0.27–0.35)	0.68	(−0.1, 0.1)
Oxygen excess factor (Ω)	3.1 (2.4–3.6)	3.1 (2.9–3.7)	0.68	(−0.9, 0.6)
Lactate	1.6 (0.9–1.9)	1.2 (0.88–1.35)	0.26	(−0.2, 0.9)
Sm–tricuspid annulus (cm/s)	3.60 (2.50–4.60)	4.08 (2.87–4.77)	0.85	(−1.56, 0.93)
Sm–basal septum (cm/s)	3.21 (2.39–4.48)	2.71 (2.13–4.66)	0.52	(−0.83, 1.53)
Sm–mitral annulus (cm/s)	5.21 (3.94–6.46)	3.98 (3.61–5.15)	0.10	(−0.24, 2.46)
Restrictive RV physiology	2, 20 %	3, 30 %	1.00	–
Ventilation time hours	141.0 (58.7–195.2)	82.0 (19.1–121.3)	0.14	(−7.7, 122.7)
Critical care stay days	7.8 (5.0–11.2)	5.0 (3.5–8.3)	0.27	(−2.3, 6.1)

All data expressed in median (interquartile range)

BTS Blalock–Taussig shunt, IVS interventricular septum, Sm systolic myocardial velocity, XC (cross clamp), SvO₂ mixed venous oxygen saturation, R.O.D. relative optical density

was initiated. Western blot analysis was performed as described previously (Lyall et al. 2000) but with modifications. Tissue was homogenised then spun at 5,000× *g* for 10 min at 4 °C. The supernatant containing the total cell protein was extracted for protein concentration assay. Samples were separated on 10 % sodium dodecyl sulfate-polyacrylamide resolving gels. Each well was loaded with approximately 30 µg of soluble protein. Molecular weight markers (Low Range, Bio-Rad) were loaded beside the

samples. Placental tissue, known to express HSP 27, was used as a reference sample and positive control on each gel. Proteins were transferred to Whatman Protran nitrocellulose membranes (Sigma). The primary antibody (HSP 27, New England Biolabs, number 2402, mouse monoclonal) was used at 1:1,000. The second antibody was horseradish peroxidase conjugated donkey anti-mouse secondary antibody (Abcam, number ab6820, 1:1,000). Proteins were visualised using the Amersham ECL detection system

(RPN2106). Bands were scanned using a Bio-Rad GS-700 imaging densitometer. Band densities were expressed relative to the density of the internal placental control sample included on every gel.

The baseline period was defined as 0–15 min after aortic cross clamp and before repeat cardioplegia administration. Baseline HSP 27 protein density was calculated from mean HSP 27 expression in the right ventricle myocardium resected during this period and considered to represent as close to the pre-operative HSP 27 expression in the myocardium. Subsequent muscle resected after this period was grouped together to determine any effect of ischemic time on HSP 27 expression.

Post-operative monitoring

Post-operatively, serum lactate level, mixed venous oxygen saturation (S_vO_2 , percent) and troponin-I were measured concurrently with echocardiography. S_vO_2 is a function of systemic arterial oxygen saturation (S_aO_2), which can be affected by various factors including right-to-left intracardiac shunt, lung pathology therefore oxygen extraction ratio [$OER = (S_aO_2 - S_vO_2)/S_aO_2$] represents a better indicator of systemic perfusion (Martin and Shekerdeman 2009). Oxygen saturation was measured from concurrent blood sampling taken from the peripheral arterial line and the central venous catheter in the superior vena cava (Martin and Shekerdeman 2009). Inotrope score was calculated (Davlouros et al. 2006) and duration of ventilation and length of ICU stay were recorded.

Statistical analysis

A statistical analysis was performed following advice from a professional statistician using the Minitab® Statistical Software Release 14.2 (Minitab, Inc., PA, USA). Non-parametric data (expressed in median + interquartile range, Q1–Q3) was analysed using the Mann–Whitney *U* test, Wilcoxon Matched Pair or Kruskal–Wallis test for continuous variables and Fisher exact test for categorical variables. The strength of relationship between two variables was tested using Spearman rank correlation (ρ). $p < 0.05$ was considered to be statistically significant.

Results

Table 1 summarises the peri-operative characteristics of the two groups. There were no significant differences between the cyanotic and the non-cyanotic group with regard to age, gender and body weight. The cyanotic group had a significantly increased haematocrit level, lower O_2 saturation and

thicker interventricular septal wall thickness. Cross clamp time and bypass time were significantly increased in the cyanotic group; however, ventilation time and length of stay in critical care were similar.

When compared to the control group with innocent murmur, TOF patients had significantly reduced systolic annular velocity pre-operatively at tricuspid (8.13 vs. 12.67 cm s^{-1} , $p < 0.0001$), septum (4.80 vs. 7.58 cm s^{-1} , $p < 0.001$) and mitral (4.83 vs. 7.52 cm s^{-1} , $p < 0.001$). Pre-operative annular velocities were similar in the cyanotic and non-cyanotic TOF groups. At post-operative day 1, systolic myocardial velocity was reduced further in the tricuspid and basal septum in both the cyanotic group ($p < 0.001$ and $p = 0.02$, respectively) and non-cyanotic group ($p < 0.001$ and $p = 0.02$, respectively) compared to pre-operative velocities. No differences in post-operative annular velocities or the presence of restrictive right ventricle physiology were found between the cyanotic and non-cyanotic group. No association was found between post-operative right ventricle velocities (tricuspid and septal) and pre-operative oxygen saturation, haematocrit, cardiopulmonary bypass time, troponin release, surgical method, degree of pulmonary regurgitation and residual right ventricle outflow tract obstruction.

All TOF patient annular velocities improved 1 week after surgery compared to day 1 (tricuspid, $p = 0.01$; septum, $p = 0.004$; mitral, $p = 0.02$) but remained below the baseline velocities of healthy children.

Post-operative troponin-I release and HSP 27

The cyanotic group released significantly more troponin-I on post-operative day 1 compared to the non-cyanotic group (Table 1). Higher troponin-I release was associated with a higher pre-operative haematocrit level ($p = 0.003$), longer aortic cross clamp time ($p = 0.02$) and cardiopulmonary bypass time ($p = 0.02$). There was no correlation between troponin I and HSP 27 or the weight of the total muscle harvested from each group.

HSP27 expression in tissue resected from right ventricle outflow tract obstruction

There was no statistical difference between the amount of ventricle muscle resected from the cyanotic compared with the non-cyanotic group (748.7 mg vs. 583.6 mg) ($p = 0.08$). Figure 1 is a Western blot showing HSP 27 expression in samples resected from two patients (numbered 9 and 19) at different times following initiation of cross clamp (patient 9 (lanes 1–7) and patient 19 (lanes 9–12)). All muscle samples collected during the aortic cross clamp period expressed HSP 27. Also shown is the internal control and the molecular

Fig. 1 Western blot showing HSP 27 expression in two different patients (patient 9 (lanes 1–7) and patient 19 (lanes 9–12) at time intervals from the start of cross clamping. Patient 9 lane: 1, 10–15 min; 2, 15–20 min; 3, 20–25 min; 4, 30–35 min; 5, 35–40 min; 6, 40–45 min; 7, 45–50 min).

Patient 19 lanes: 9, 5–10 min; 10, 10–15 min; 11, 15–20 min; 12, 20–25 min. Internal control human placenta tissue known to express HSP27 (IC). Molecular weight markers are shown on the left; 31.25 µg total myocardial protein was loaded per lane

weight markers used to confirm the HSP 27 protein molecular weight was the expected 27 kDa.

When both cyanotic and non-cyanotic groups were combined and analysed for HSP 27 levels before 15 min (baseline) or after 15 min, there were no differences between any of the groups (Fig. 2a). The results for median and interquartile range for HSP 27 expression were as follows: (1) all samples collected during the first 15 min of aortic cross clamp time were 0.75 (0.64–0.93), samples collected after the first 15 min were 0.74 (0.58–0.93) and for all time points together, 0.74 (0.61–0.93). Next, a separate analysis was performed for the cyanotic and non-cyanotic groups separately. The results are shown in Table 1 and Fig. 2b. There was a significant increase in HSP 27 levels in the combined <15 min and >15 min cyanotic groups compared to the combined <15 min and >15 min non-cyanotic groups ($p=0.01$). When the <15 min and >15 min time points were analysed separately, there was a significant increase in HSP 27 levels in the <15 min cyanotic compared to the <15 min non-cyanotic group ($p=0.03$). There was no significant relationship between baseline HSP 27 levels and pre-operative variables, i.e., age, weight, oxygen saturation, haematocrit and right ventricular wall thickness.

In the cyanotic group, baseline HSP 27 levels (tissue collected during the first 15 min of aortic cross clamp (and the closest representation of pre-operative status) correlated highly with oxygen extraction ratio. The results are shown in Fig. 3a ($p=0.028$). In contrast, this relationship was not seen in the non-cyanotic group ($p=0.34$). In the cyanotic group, baseline HSP 27 levels also significantly correlated with post-operative basal septal velocity (Fig. 3b, $p=0.036$), and again, this relationship was not seen in the non-cyanotic group ($p=0.93$). There was no correlation of HSP 27 with the tricuspid or mitral annular velocity. Figure 3c shows that HSP 27 also significantly correlated with better mixed venous oxygen saturation in the cyanotic group ($p=0.02$) but

not the non-cyanotic group ($p=0.93$). None of the above associations were found when the analyses were performed on tissues collected after 15 min from the start of cross clamping time. No other associations were found.

Discussion

Although TOF can present with a wide spectrum of clinical and anatomical variations, the tetrad of anatomical defects that are prominent in the condition are right ventricular outflow tract obstruction, interventricular communication, biventricular origin of the aortic valve and right ventricular hypertrophy. Early and late morbidity is related to the function of the right heart which is exposed to increased pressure pre- and post-operatively. A larger VSD and outflow tract obstruction subject the right ventricle to chronic pressure overloading prior to surgery resulting in compromised function and slower recovery after repair (Wernovsky et al. 1995). Abnormal post-operative load within the ventricle which can be due to residual lesions or pulmonary regurgitation can lead to right ventricle dilation, right heart failure, conductance disturbance, dysrhythmias and sudden death. Thus, despite surgery, the patients may not be fully cured. This had led, over the last 50 years, to an expanding adult population with compromised right heart function (Davlouros et al. 2006).

This study is important since it is one of few that have investigated HSP levels in human immature myocardium. The study was powered based on previous studies from our group (Peng et al. 2011). The major finding was that cyanotic TOF myocardium baseline HSP 27 levels (which close as possible represents pre-surgery status) were significantly increased compared to the non-cyanotic group and was also associated with better cardiac output/contraction. Thus, HSP 27 may be important in myocardial protection. Patients in

Fig. 2 **a** Box and whiskers plot (median, interquartile range) of HSP 27 (relative optical density (R.O.D.)) expression in all patients (cyanotic and non-cyanotic) grouped by time interval collected during aortic cross clamping time ($p>0.05$). **b** Box and whiskers plot (median, interquartile range) of HSP 27 (R.O.D.) expression in the cyanotic (left panel) and non-cyanotic patients (right panel) grouped by time interval collected during aortic cross clamping time; ($*p<0.05$)

the cyanotic group have lower oxygen saturations and are more vulnerable than non-cyanotic patients. Interestingly, this increase in HSP 27 was not apparent in the tissue collected and combined after 15 min until the end of surgery (typically 15–45 min). Whether this relates to protein stability, the surgical procedure or some other factor remains to be elucidated. Other possibilities are that as surgery time advances other protective mechanisms such as other protective HSPs are increased to maintain protection. Also, prior to surgery, the myocardium from the cyanotic and non-cyanotic groups was exposed to different conditions (<15 min group), whereas patients in the >15 min group are all now exposed to the same oxygen environment. Thus,

even though after 15 mins the HSP 27 levels are reduced, it may be that post-operative function is improved because of other protective mechanisms being switched on during the cross clamp time; these would include other protective HSPs or it may be that repair mechanisms have been set in place by the increase in HSP 27 (to repair protein folding), and once this happens, the HSP 27 declines.

An association with HSP 27 was found with one out of the three echo ventricular function parameters (septal region) but not in the right or left ventricle free wall. This may be linked to the fact that the majority of muscles is removed from the septal area.

Despite a similar reduction in RV function in both groups, the cyanotic group had higher troponin levels. Possible explanations are the longer cross clamp and bypass time, more muscle resected, reduced myocardial protection or susceptibility to injury in the cyanotic group. Pediatric patients undergoing cardiac surgery have been shown to demonstrate greater susceptibility to ischemia–reperfusion injury and worse clinical outcome when cyanotic (Bulutcu et al. 2002; Modi et al. 2002).

HSP 27 is one of the most widely distributed of the small HSPs (Ghayour-Mobarhan et al. 2012). Animal and cell culture models have shown that the roles of HSP 27 include protein chaperone function, regulation of glutathione levels and apoptosis (Arrigo et al. 2007) and heart tube formation (Brown et al. 2007). Intracellular HSP 27 protects cells against external stress such as heat shock, mechanical and oxidative stress (Carper et al. 1997). HSP 27 is also thought to be involved in an autoimmune response (Ghayour-Mobarhan et al. 2012). Like other small HSPs, HSP 27 is regulated at both the transcriptional and posttranslational level. HSP 27 is phosphorylated by MAPKAP kinase 2 as a result of the activation of the p38 MAP kinase pathway (Arrigo et al. 2007). Since phosphorylated HSP 27 is linked to actin–myosin interactions, it would be of interest in future studies to assess phosphorylated HSP 27 and sarcomere structure in TOF.

Little is known about the role of HSP 27 in the immature human heart. Most of the current knowledge has come from animal models or limited studies on adult cardiac disease. HSP 27 expression has been shown to protect against myocardial infarction (Willis and Patterson 2010; Efthymiou et al. 2004) and is increased in the rat left ventricle myocardium after coronary ligation and in human patients with dilated cardiomyopathy (Knowlton et al. 1998). Studies on HSP 27 knockout mice have shown that, at least in this species, HSP 27 is not essential for cardiac development but plays a role in the antioxidative response during ischemia–reperfusion

Fig. 3 **a** Scatter plot of oxygen extraction ration and baseline HSP 27 in cyanotic patients (left) and non-cyanotic (right). **b** Scatter plot of post-operative (day 1) basal septal velocity and baseline HSP 27 in cyanotic patients (left) and non-cyanotic (right). **c** Scatter plot of mixed venous oxygen saturation and baseline HSP 27 in cyanotic patients (left) and non-cyanotic (right)

injury (Willis and Patterson 2010). HSP 27 delivered with a virus vector into neonatal rat cardiomyocytes was shown to protect against apoptosis as well as against hypoxic stress (Brar et al. 1999), and modest increases in HSP 27 have also been shown to protect against ischemia reperfusion injury in dogs (Willis and Patterson 2010). These studies suggest that HSP 27 plays a role in antioxidant mechanisms during ischemia reperfusion injury.

Studies on the cardioprotective roles of other HSPs in right heart function are limited but suggest HSP 32 and 72 may be cardioprotective in the right heart (Willis and Patterson 2010; Peng et al. 2011; Nakamura et al. 2000). As well as HSPs, it may be that other mediators of delayed ischemic preconditioning may occur in the myocardium, and these will be the focus of future investigation. These may include investigation of antioxidant enzymes such as superoxide dismutase, nitric oxide synthase and cyclooxygenase (Eisen et al. 2004).

Drugs are now used to increase cellular HSPs as therapy for neurodegenerative diseases which are caused by misfolded proteins (Nakamura et al. 2000). Such drugs, gene therapy procedures as well as remote pre-conditioning to increase HSPs prior to surgery, may one day be used in congenital heart disease in children. Before this can happen, more studies such as the one herein are required to fully understand the role of HSPs in TOF.

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