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Chapter 14

THE OXIDATIVE STRESS MECHANISMS ASSOCIATED WITH MYOCARDIAL INJURY FOLLOWED BY HEART FAILURE IN PEDIATRIC PATIENTS UNDERGOING SURGERY FOR CONGENITAL HEART DISEASE

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ABSTRACT

Congenital heart disease (CHD) affects 6 to 8 infants per every 1,000 live births and remains a significant cause of infant death. Recent advances in surgical techniques, which are associated with enhanced myocardial preservation methods, have improved the outcome in these cases. However, progressive ventricular dysfunction remains one of the leading causes of death during the postoperative course. While the mechanisms are not fully understood, ischemic factors associated with oxidative stress mechanisms have been suggested. Multifocal areas of myocardial injury seem to be the cause of heart failure for infants who do not survive beyond the perioperative period. They were described in patients submitted to surgery for CHD with and without cardiopulmonary bypass (CPB) and in patients who died from CHD prior to surgical treatment. Contraction band necrosis and dystrophic calcification were found in most of the infants who had undergone surgery with CPB. Coagulation necrosis and healing were observed in infants who had

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undergone surgery without CPB. Infants with no surgical intervention mainly exhibited intracellular edema, which is a lesion considered to be a histological marker of congestive heart failure. The morphological aspects of these lesions suggest ischemia as the main cause. However, the presence of contraction band necrosis in patients who had undergone CHD repair with CPB suggests the additional mechanism of ischemia/reperfusion (IR). Oxidative stress mechanisms were directly related to these particular types of myocardial injuries. Importantly, 4-hydroxinonenal (4-HNE), a marker of lipid peroxidation, is strongly expressed, especially in irreversible myocardial lesions. This finding suggests that 4-HNE may be the predominant oxidative stress mechanism that occurs in these patients. While the exact mechanism is not fully understood, it has been suggested that endogenous catecholamine release could have a role in this process. Although morphologically similar, myocardial injuries observed in patients with CHD and CPB, which evolved with sepsis in the peri-operatory period exhibited a completely different set of oxidative stress mechanisms. Increased concentrations of nitrotyrosine protein adducts were observed in these patients, suggesting that peroxynitrite-mediated protein nitration may be the predominant oxidative stress mechanism found in these situations. This confirms previous observations in humans that argue against an ischemic origin for sepsis-induced cardiac injury. Some authors have suggested that a dysfunctional microcirculation and direct myocardial cytotoxicity of the products of septic shock and the actions of various mediators of sepsis could be implicated as causative agents in these cases. This chapter emphasizes the essential role of the myocardial injuries in infant patients with CHD who undergo surgical repair techniques that includes CPB. The underlying mechanisms of these lesions seem to be related to the development of ischemia or ischemia/reperfusion followed by oxidative stress mechanisms that vary depending on whether sepsis was present. These findings warrant further studies.

Keywords: Congenital heart disease; ischemia; sepsis/septic shock, myocardial injury; infants; oxidative stress; lipid peroxidation

INTRODUCTION

Congenital heart disease (CHD) is among the most frequently observed of all major birth defects. CHD is characterized by a gross structural abnormality of the heart or of the intrathoracic great vessels that is potentially of functional significance (Mitchell et al., 1971). CHD affects 6 to 8 infants per every 1,000 live births and is the most significant cause of death in the first year of life (Hoffman *and* Kaplan, 2002). Survival into adulthood depends on the nature of the malformation and on the nature of the medical intervention, particularly when palliative or corrective surgical proceduresare involved (Gatzoulis et al., 1999).The aim of this chapter is to describe the most frequent myocardial injuries that lead to the death ofthese patients after the repair of a CHD at the University Hospital of Ribeirão Preto, Brazil. The presence of the mechanisms of oxidative stress in the injured myocardium of these patients is discussed.

ETIOLOGY

The etiology of congenital heart disease is still unknown. Only 15% of cases of CHD can be assigned to a known cause (Nora et al., 1991). Some types of CHD can be related to chromosome or gene defects, environmental factors or a multifactorial etiology (Nora *and* Nora, 1976).Only2% of all cases of CHD can be attributed to known environmental factors. Risk factors,such as maternal insulin-dependent diabetes mellitus and phenylketonuria, are well known as two of the leading causes of CHD. Other reported risk factors include maternal obesity, alcohol use in pregnancy, rubella infection, febrile illness, use of such drugs as thalidomide and retinoic acid, and exposure to organic solvents and lithium (Kuciene *and* Dulskiene, 2008).

MORTALITY

Mortality occurs mainly in patients with severe forms of CHD requiring prompt surgical intervention (Kern et al., 1998). Interestingly, the relative contribution of the causes of death in patients with CHD has changed over time. Arrhythmia followed by congestive heart failure had been considered the main contributing cause of death. However, the mortality figures collected over the past decade showed an increaseinmyocardial infarction as the cause of death (Pillutlaet. al., 2009).The development of severe sepsis after cardiac surgery, which is associated with multiple organ failure, has also increasingly factored into the mortality rate (Thijs et al., 1996).

CARDIAC SURGERY

Cardiac surgery developed slowly in the 1940s when there were only a few types of surgical procedures that could be performed without the use of cardiopulmonary bypass: closure of a patent ductus*(Gross and Hubbard, 1939)*,coarctationof aorta repair*(*Crafoord *and* Nylin, 1945),the Blalock-Taussig shunt *(*Blalock *and* Taussig, 1945),and mitral commissurotomy (*Bailey,* 1949). In the early 1950s,the closure of atrial septal defects with the use of hypothermiawas developed(Lewis *and* Taufic, 1953), but it was obvious to the surgeons of this era that a heart-lung machine would be required to deal with the majority of CHD and valvular heart diseases (Stoney, 2009). Since that period, advances in surgical, anesthetic, and cardiopulmonary bypass techniques have led to significant improvements in diagnosis, surgical repair and postoperative management (Jones *and* Elliott, 2006). In fact, there is a trend toward earlier repair of congenital cardiac defects in neonates and infants (Drinkwater *and* Laks,1993). However, perioperative myocardial damage remains the most common cause of morbidity and death following the technically successful surgical correction of a CHD (Bull et al., 1984; Taggart et al., 1997; Modi et al., 2002; Elias *and* Souza, 2004).

ANESTHESIA

For infants undergoing surgery, the length of exposure to anesthetic agents depends on the nature of the surgical procedure. Surgical repair for complex cyanotic heart lesions, such as hypoplastic left heart syndrome and the transposition of the great arteries, requires 4–6 h of general anesthesia at most institutions (Wise-Faberows *and* Loepke, 2011).At the University Hospital of Ribeirão Preto, anesthesia for pediatric cardiac surgeries is performed according to the customary protocols. Midazolam (0.1–0.2 mg/kg) and fentanyl (50–100 μg/kg) are the anesthetic agents most frequently utilized.Various anesthetics may lead to alterations in cardiac pathology, but midazolam and fentanyl are not known to aggravate myocardial lesions. Fentanyl, an opioid agonist, has reportedly enhanced the recovery of mechanical function after ischemia (Kato et al., 2000), and midazolam is unlikely to affect in vivo ROS formation (Kevin et al., 2005).

CARDIOPULMONARY BYPASS (CPB)

Cardiopulmonary bypass (CPB) is a generally necessary procedurein cardiac surgery in neonates and infants. CPB, however, causes profound alterations in physiological fluid homeostasis (Hirleman *and* Larson, 2008). The age and size of the patient, the underlying cardiac pathology and the type of surgical techniques influence what perfusion methods are chosen and the construction of the CPB circuit (Jones *and* Elliott, 2006).Despite significant improvements, CPB remains a non-physiological procedure. The effects of hypothermia, altered perfusion, hemodilution, acid-base management, embolization and the systemic inflammatory response have been challenging, particularly for neonates and infants. These challenges are primarily related to the smaller circulatory volume, the immaturity of most organ systems and the increased capillary membrane permeability of neonates and infants (Jonas et al., 2003; Jones *and* Elliott, 2006). Moreover, cardiomyocytes can be affected by hypoxic conditions, and the ischemic effects can induce rapid or gradual changes inthe membrane systems that cause reversible or irreversible injury (Asano et al., 2003). Experimental studies of myocardial ischemia and reperfusion have established that reperfusion also has negative consequences during circulatory interruption (Follette et al., 1981; Buckbergand Allen, 1995). Due to the necessary interruption in coronary circulation required by nearly all cardiac surgeries, the potential for reperfusion damage is significant. If a reperfusion injury does occur, the initial damage may contribute to the impaired cardiac performance that develops immediately after surgery that may then lead to myocardial fibrosis (Kirklinand Barratt-Boyes, 1993; Castañeda et al., 1994).

THE IMMATURE HEART

The immature heart with a higher water and protein content per gram is a denser structure than the adult heart. As a result of the structural differences, the immature heart is less compliant and has less preload reserve (Billingsley et al., 1991). The immature myocardium exhibits a lower rate of maximum tension development and a greater coupling of the two

ventricles (Teitel et al., 1983). The normal neonatal heart also has a reduced inotropic reserve and operates under maximal adrenergic stimulation. Because of these differences, the immature myocardium exhibits a greater negative inotropic response to common anesthetic drugs (Boudreaux et al., 1984) and a less predictable positive inotropic response to adrenergic agonists, such as epinephrine (Caspiet al., 1991).

MYOCARDIAL INJURIES

While the neonatal myocardium is relatively resistant to ischemia, it has a limited functional reserve and has a greater susceptibility to the effects of increased afterload (systemic vascular resistance) (Jones *and* Elliott, 2006). In addition to differences in the structure, function, and biochemistry of the healthy immature and mature heart of infants and children,heart failure in CHD can be due to volume/pressure overload caused by shunts and obstructive lesions of the heart (Hammon, 1995; Chaturvedi *and* Saxena, 2009). Transient myocardial ischemia may occur during anesthesia and surgery. Myocardial performance is compromised to a variable degree after ischemia and subsequent reperfusion. This dysfunction reflects either irreversible (infarction) or reversible injury (Kato et al., 2000). Within hours after an infarction, the infarcted muscle loses its striations, and changes in its staining properties have been reported (Mallory et al., 1939).Within 24 h, 94% of human infarcts have wavy fibers that are indicative of intercellular edema, and 90% have clear necrosis, which is characterized by altered staining properties and nuclear pyknosis or karyolysis. By the fourth or fifth day, the removal of dead muscle is clearly observed (Mallory et al., 1939; Fishbeinet al., 1978).

HISTOPATHOLOGIC EVALUATION OF MYOCARDIAL INJURIES

Colliquative Myocytolysis (CM)

It is defined as a progressive loss of myofibrils paralleled by intramyocellular edema (Baroldi et al., 1998). This process starts around apparently normal nuclei with myofibrillar disappearance producing an increasing vacuolization of myocardial cells until a histologic pattern of empty sarcolemmal tubes, without any cellular reaction or signs of healing results. This lesion is generally present in the subendocardial half of the cardiac wall and is considered as the histological hallmark of congestive heart failure (Turillazzi et al., 2005); (Figure 1A).

Hemodynamic Alterations

Intercellular edema, hemorrhage, and congestion can be observed in the injured myocardium (Figure 1B).

Contraction Band Necrosis (CBN)

A plurifocal lesion characterized by a hypercontraction of the myocardial fibers with the formation of abnormal transverse bands (Baroldi, 1975). Considered as a marker of cell death, associated with catecholamine excess that can be identified a short time after irreversible myocyte injury (Virmani et al., 1996), it may generally occur in all those different conditions, resulting in alterations of the interaction between calcium and catecholamines (Arnold et al., 1985; Rump et al., 1995). CBN is a consequence of reperfusion in general or to a temporary vascular spasm (Braunwald, 1990); (Figure 1C).

Coagulation Necrosis (CN)

A monofocal lesion which starts in irreversible relaxation of myocardial cells stretched by the intraventricular pressure/pulsation as shown by elongation of sarcomeres and nuclei without other early changes. Later, a polymorphonuclear leukocytic infiltration followed by macrophagic digestion and healing is the repair phases (Baroldi et al., 1998); (Figure 1D).

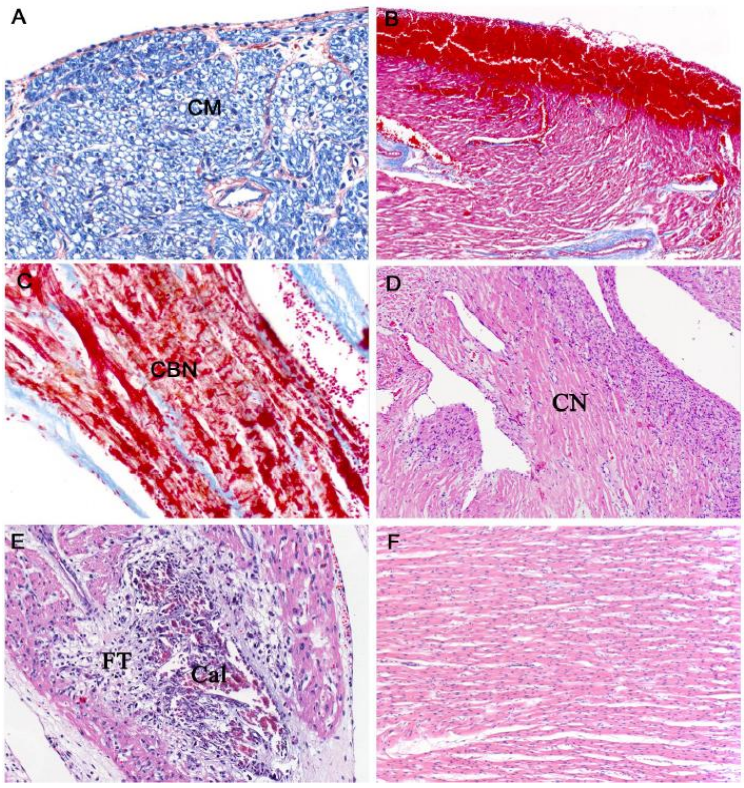


Figure 1. The histopathology of the principal injuries observed in the myocardium of infants with congenital cardiac heart disease and repair leading to death. A: Severe colliquativemyocytolysis (CM) in the subepicardial region; PTAH,400x. B: Extensive hemorrhagia in the subepicardial region; HE, 100x. C: Area of contraction band necrosis observed in the myocardium; Azan, 320x. D: Area of coagulation necrosis(CN) observed in the myocardium, HE, 100x. E: Dystrophic calcification associated with fibrous tissue into the myocardium (FT); HE, 200x. F: Control myocardium; HE, 200x.

Fibrous Tissue (FT)/ Healing Myocardium (HM)

The presence of myocardial fibrosis is an important aspect of heart failure and an index of poor prognosis. Cardiac fibrosis can be defined as progressive accumulation of extracellular matrix (ECM) components like collagens I, III, IV, laminin, fibronectin within the myocardium (Herpel et al., 2006). Fibrosis can be reparative or reactive; "reactive" fibrosis, in which collagen accumulates in perivascular and interstitial tissue, is not accompanied by myocyte loss while in "replacement (reparative)" fibrosis myocyte loss and secondary microscopic scarring is detected (Mann, 2008); (Figure 1E).

Calcification (Cal)

Calcium deposition at sites of inflammation and necrosis is a fundamental, but poorly understood element of the response of tissue to injury. Calcification, confined to or mainly involving the myocardium is most often found in old ischemic infarcts. It has also been noted in patients after cardiovascular surgery and is seen with sufficient frequency at necropsies (Hermann et al., 1963; Ivandic et al., 1996); (Figure 1F).

OXIDATIVE STRESS

Reactive Oxygen Species (ROS): 4- Hydroxynonenal

During cardiac surgery, the heart is kept in an arrested state for a substantial period of time and is subjected to reperfusion. Because this condition is analogous topostischemic reperfusion, the possibility of the development of ROS-mediated injury during cardiac surgery has been considered (Menasche et al., 1986; Johnson et al., 1987). ROS are oxygen-based molecules characterized by high reactivity. ROS include free radicals and non-radicals that are capable of generating free radicals (e.g., hydrogen peroxide (H^2O^2)). If present in excess, free radicals can induce oxidative damage to DNA strands, proteins, lipids and other molecules. Free radicals can also lead to hypertrophy, apoptosis/cell death, and intracellular Ca^{2+} overload in myocytes (Weisfeldt, 1988; Josephson et al., 1991; Sawyer et al., 2002; Chen et. al., 2003; Nakamura, 2005). ROS also has been implicatedin the damageof lipid cell membranes through the process of lipid peroxidation. In this process, several aldehydes are generated, including 4-hydroxy-2-nonenal (HNE),which is recognized as the most reliable marker of lipid peroxidation.With its intense lipophilic properties, HNE tends to concentrate in the biomembranes where phospholipids and proteins, such as transporters, ion channels and receptors, can quickly react with it (Esterbauer, et al., 1991; Toyokuni, 1999; Uchida, 2003; Poli et al., 2008).

Reactive Nitrogen Species (RNS): 3-Nitrotyrosine

Tyrosine-nitrated proteins have been broadly observed in both the normal and diseased cardiovascular system. Nitrated proteins have been detected with a variety of techniques and have been most notably observed in the intravascular space, the vessel wall and the myocardium (Bartesaghi et al., 2007). Nitrotyrosine accumulation reflects a loss of balance between oxidant formation and antioxidant defense mechanisms,which were formerly known as oxidative stress (Sies, 1991).The nitration of biomolecules, such as proteins and lipids,are biologically significant and are largely dependent on nitric oxide (•NO)-derived oxidants (Radi, 2004). The toxicity of nitric oxide (NO) is enhanced by its reaction with superoxides to form peroxynitrite ($ONOO^-$). Peroxynitrite may decompose into oxidants that create nitrotyrosine, which is a process detected *in vivo* and in various human diseases, including myocardial ischemia (Beckman andKoppenol, 1996; Xie *and* Wolin, 1996; Weinstein et al., 2000).

OXIDATIVE STRESS MECHANISMS ASSOCIATED WITH MYOCARDIAL INJURY FOLLOWED BY HEART FAILURE IN PEDIATRIC PATIENTS UNDERGOING SURGERY FOR CHD

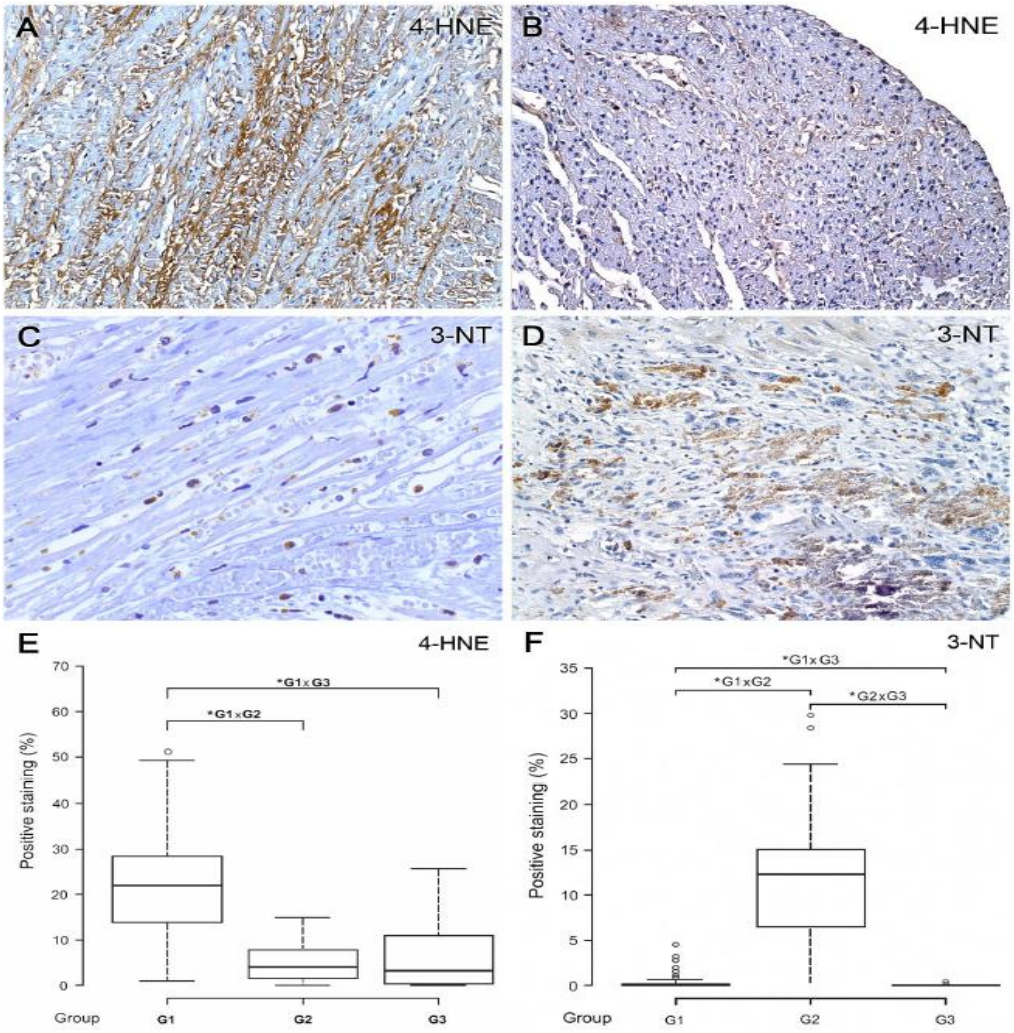
In a recent study, Oliveira et al. (2011) demonstrated that HNE was strongly expressed, especially in irreversible myocardial lesions, suggesting that lipid peroxidation may be the predominant oxidative stress mechanism found in postsurgical patients. The processes that predispose the ischemic heart to toxicity by HNE and related species are not fully understood (Haenen et al., 1989; Schömig 1990; Blasig et al., 1994; Eaton et al., 1999; Kaminski et al., 2008), but the release of endogenous catecholamines may be a factor.The excessive release of catecholamines has been reported to be responsible for the development of significant cellular damage, myocyte death in heart failure and myocardial infarction (Carelockand Clark, 2001; Goldspink, 2003; Ueyama et al., 2003). The contribution of catecholamines to stress-induced heart injury has been recognized for more than 40 years (Raab et al., 1968). Catecholamines can easily undergo oxidation with the production of unstable catecholamines-O-quinones. This process subsequently creates adrenochromes and initiates the subsequent production of oxygen free radicals, such as superoxide radicals. The superoxide radicals can be reduced by superoxide dismutase to hydrogen peroxide, which damages the membrane integrity. Superoxide radicalshave been suggested to be responsible for the damage observed in catecholamine-induced cardiomyopathy (Behonick et al., 2001; Tappia et al., 2001). Experimental models using a synthetic catecholamine (isoproterenol) have demonstrated that higher demands can produce alterations in the cellular redox state and lipoperoxidation in cardiomyocytes (Neri et al., 2007), compromising structural proteins that form the dystrophin–glycoprotein complex (Campos et al., 2008). The pathogenesis of isoproterenol cardiac injury appears to be multifactorial, and the process is not fully understood. The process involves the initial adrenergic overstimulation of the myocardium with a concomitant decrease in the myocardial blood supply due to peripheral vasodilatation followed by microthrombus formation with apparent obstruction of many small vessels (Blasig et al., 1985; Rona, 1985, Diaz-Munoz, 2006). Such derangement is associated with myocardial

ischemia and the consequent necrosis. The re-establishment of blood flow in the ischemic myocardium is a common situation in cardiovascular surgery. The reintroduction of an abundant supply of oxygen at the onset of reperfusion initiates a burst of ROS within the first few minutes of reflow. The ROS causes peroxidation of membrane phospholipids,resulting in damaged membrane integrity, intracellular calcium overload, and cell death (Singal et al., 1981; Josephson et al., 1991). This result has been demonstrated both experimentally and in patients undergoing open-heart surgery (Jennings, and Reimer, 1983; Kim et al., 1994).

Although morphologically similar, myocardial injuries after CHD repairs that include the use of CPB, which evolved with sepsis in the peri-operatory period showed a completely different profile of oxidative stress mechanism.Hospital-acquired infections are a major cause of morbidity and mortality for pediatric patients undergoing cardiac surgery. The development of these infections affects the success of the surgery itself and affects postsurgical outcomes for these children (Levy et al., 2003). Sepsis is the clinical manifestation of the immune and inflammatory response arising from infection.The most common cause of such infections is the contamination of the blood with bacteria. Severe sepsis is defined as sepsis with organ dysfunction. Septic shock is sepsis with severe hypotension and a decrease in perfusion to the critical organ systems despite increased circulating levels of endogenous catecholamines (Bone et al., 1992; Salvemini *and* Cuzzocrea, 2002). Septic shock is a subset of severe sepsis and is associated with a downregulation of the β-adrenergic response to catecholamines. It is likely that several mechanisms are responsible for this phenomenon (Shepherd et al., 1987; Bone et al., 1992). A dysfunctional microcirculation and direct myocardial cytotoxicity of the products of septic shock, along with the actions of the various mediators of sepsis, have been implicated as causative agents in adults and children (Parrillo et al., 1985, Turner et al., 1999). In septic shock, cardiodepression may be induced through oxidative stress from the secretion of proinflammatory mediators or from nitrosative stress (Muller-Werdan et al, 1997). Infants with CHD who have undergone a surgical repair that included the use of CPB showed increased concentrations of nitrotyrosine protein adducts in the setting of sepsis/septic shock. This finding suggests that peroxynitrite-mediated protein nitration may be the predominant oxidative stress mechanism found in this setting . The secondary products of oxidative stress, such as HNE (4-hydroxynonenal), are normally found in patients that have not developed sepsis/septic shock. These observations are summarized in Figure 2.

CONCLUSION

Significant advances in clinical expertise and technique have enabled complex neonatal cardiac surgery to be routinely undertaken (Jonesand Elliott, 2006), but the variability in cardiac pathology and surgical techniques associated with perioperative myocardial injuries requires a special focus on the immature myocardium. The lipid peroxidation of 4-HNE was the principal mechanism of oxidative stress, accounting for the myocardial lesions observed in patients with CHD who had undergone surgery with CPB. An increased concentration of 3-nitrotyrosine (3-NT) protein adducts was observed in the septic hearts of infants, suggesting that protein nitration could be the predominant oxidative stress mechanism found in septic patients. The true significance of these findings from autopsy requires further clinical study.



A: Extensive area of the myocardium showing strong expression of 4-HNE (G1 group), 200x. B: 4-HNE expression observed in sepsis/septic shock patients (G2 group), 200x. Note that 4-HNE is significantly increased in non-septic patients. C: The myocardium of G1 group showed only a slight expression of nitrotyrosine, 400x. D: On the contrary, the myocardium of G2 group showed a strong expression of nitrotyrosine in an intramural band of the injured myocardium, 200x. E: Boxplot for optical density by image analysis demonstrated 4-HNE. Note that G1 is significantly augmented in comparison with G2 and G3. No significant differences were noted between G2 and G3. F: Boxplot for optical density by image analysis demonstrated nitrotyrosine. Note that G2 is significantly augmented in comparison with G1 and G3, and G1 is augmented in comparison with G3. Std. Dev.: Standard Deviation. Significant differences ($P<0.05$) between groups are represented by an asterisk (*). G1: CHD surgical repair without sepsis/septic shock complications, G2: CHD surgical repair with sepsis/septic shock complications, G3: Control.

Figure 2. An assessment of oxidative stress measuring the extent of myocardial 4-HNE and nitrotyrosine immunoreactivity in patients with congenital heart disease (CHD) who had undergone a surgical repair with cardiopulmonary bypass (CPB) that led to death. A, C, E: CHD and CPB without sepsis; B, D, F: CHD and CPB with sepsis.

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