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Hypoxia-Induced Molecular and Cellular Changes in the Congenitally Diseased Heart: Mechanisms and Strategies of Intervention

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Abstract

Tissue hypoxia plays a critical role in the pathobiology of congenital heart diseases, especially with regard to cyanotic patients. Here, we describe the cellular and molecular mechanisms induced by hypoxia in the diseased heart, with particular attention to the metabolic and functional changes that underlie the hypoxia-induced right ventricle remodelling. The role of reactive oxygen species in transcriptomic changes, DNA damage, contractile dysfunction and extracellular matrix remodelling will be addressed. Furthermore, the reoxygenation injury, which occurs when oxygen is reintroduced upon initiation of cardiopulmonary bypass, will be discussed. This allows a better understanding of the risks associated with the reoxygenation injury in children undergoing open-heart surgery and helps to improve strategies of intervention for myocardial protection.

Keywords: hypoxia, congenital heart disease, cyanosis, reoxygenation injury, cardiovascular disease

1. Hypoxia in cardiovascular disease and congenital heart disease

The term hypoxia refers to a condition where the tissues are not adequately oxygenated, usually due to interrupted coronary blood flow or a reduction in arterial blood oxygen partial pressure [1]. With the heart being a highly oxidative organ, relying on high oxygen consumption for the work of its contractile machinery, it appears obvious that cardiac cells are very sensitive to oxygen deprivation [2]. Heart hypoxia, which originates as a

result of disproportion between the amount of oxygen supplied to the cardiac cell and the amount required by the cell, plays a critical role in the pathobiology of several cardiovascular diseases [3]. These include myocardial infarction, coronary artery diseases, heart failure secondary to pulmonary disease and congenital heart diseases [1, 4, 5]. In patients with coronary artery diseases and myocardial infarction, hypoxia is usually due to the formation of an atherosclerotic plaque in the wall of coronary arteries, which reduces the perfusion of myocardial tissue [6]. In addition, a rupture of the plaque might result in complete arterial occlusion, leading to the death of the ischemic tissue [6]. The increased O_2 consumption caused by pressure overload and reduced O_2 delivery, due to impaired coronary blood flow, are the main causes of hypoxia in patients suffering from heart failure secondary to pulmonary hypertension [7].

The scenario looks different when shifting the focus to myocardial hypoxia in paediatric patients with congenital heart diseases (CHDs). Diseases affecting the heart, in fact, have usually a different pathophysiology in children compared to adult population [8]. Furthermore, as a result of the different pathophysiological function of the defective heart, the paediatric and adult patients are differently susceptible to stress insults, although there is still disagreement on whether the vulnerability of immature heart is less or more than for adult heart [9–12].

Congenital heart diseases include a wide spectrum of anomalies of the cardiac architecture, and they are usually classified based on the anatomical and pathophysiological nature of the defect. The main anomalies involve atrioventricular junctions and valves [i.e. atrial septal defect (ASD), ventricular septal defect (VSD), atrioventricular septal defect (AVSD)], the ventricular outflow tracts [like in tetralogy of Fallot (TOF)] or can consist of univentricular hearts [like single ventricle (SV)] [13].

More often, congenital heart defects are simply classified as cyanotic and acyanotic, depending on whether or not the defect affects the amount of oxygen in the body. In cyanotic heart defects, as consequence of a mixture between oxygenated and de-oxygenated blood, less oxygen-rich blood reaches the different tissues of the body, resulting in a bluish skin, lips and nail bed colour. This category includes defects such as TOF, transposition of the great vessels or truncus arteriosus. On the other hand, non-cyanotic CHD patients do not experience a lack in blood oxygen supply; therefore, they rarely develop the bluish colour, except for few occasion, when the baby needs more oxygen, such as when crying and feeding. Atrial and/or ventricular septal defects or coarctation of the aorta are examples of acyanotic CHDs [14, 15].

Several studies have shown that, among CHDs, cyanotic patients are much more prone to develop a severe chronic hypoxia state, compared to the acyanotic ones, as the lack of oxygen exposes the cardiac tissue to an increase in free oxygen radicals [16, 17]. Therefore, when considering the treatment of these patients, the oxidative stress problem has to be taken into account, in addition to the other anomalies that characterize these defects. Nevertheless, care must be taken also for the treatment of acyanotic patients, to prevent the hypoxia that might develop in a later stage.

2. Mechanism underlying the hypoxia response in Congenital Heart Disease

2.1. Depletion of antioxidant defences

The exposure of a defective heart to chronic hypoxia induces molecular and cellular changes that affect the myocardial function and metabolism. One of the most typical sign of a heart-developing chronic hypoxia is the unbalance between the level of reactive oxygen species (ROS) and the antioxidant defence system. ROS are physiologically produced during cell metabolic and energetic reactions [18]. Nevertheless, the body is endowed with antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase and vitamins (retinoic acid, alpha-tocopherol, ascorbic acid) that can counteract this physiological production [19]. Even in case of excessive free radical production, the body responds to restore harmony balance [20]. However, under chronic hypoxia, a downregulation of antioxidant defences occurs, making the cells vulnerable to oxidant damage. Two different studies analysing the oxidant status of paediatric patients with CHDs revealed that the oxidative stress index, given by the ratio between pro-oxidants and antioxidants factors, was higher in the plasma of cyanotic children compared to the controls [16, 17]. No difference was found between acyanotic and control groups, thus confirming that the anatomical defect dictates the hypoxic level and the oxidative status [16, 17].

2.2. Hypoxia-induced metabolic and functional changes: the basis of right ventricle remodelling

Metabolic markers of oxidative stress, such as 8-isoprostane, were shown to be high in cyanotic patients' heart as revealed by our study evaluating the transcriptomic analysis of patients with tetralogy of Fallot (TOF) [21]. In a different study, we performed a genome-wide investigation to determine the global gene expression profiles associated with chronic hypoxia in the heart of patients with TOF, undergoing corrective cardiac surgery. The data revealed that 795 genes were differently expressed in cyanotic versus acyanotic hearts. In particular, genes associated with the contractility machinery function and MAPK signalling, involved in cell survival and antioxidant defence, were downregulated, whereas growth, remodelling and apoptosis-related genes were upregulated in the cyanotic group compared to the acyanotic one [22].

The altered gene expression triggered by the rise in reactive oxygen species is mostly responsible for the cellular and molecular changes that affect the myocardial function and metabolism, thus predisposing the heart to hypertrophy and failure. The hypoxia-induced downregulation of the sodium-calcium (Na^+–Ca^{2+}) exchanger (NCX1) in cyanotic patients decreases myocyte calcium handling capacity, leading to mechanical dysfunction [22]. In addition, ROS can induce oxidative modification of the sarcoplasmic membrane channels: the ryanodine receptor2 (RyR2) becomes abnormally activated while sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) is inhibited, causing an abnormal Ca^{2+} transient between cytosol and sarcoplasmic reticulum that contributes to the cardiomyocytes contractile dysfunction [23, 24]. ROS accumulation has also a detrimental effect on mitochondrial function by sustaining

mitochondrial permeability transition pore (mPTP) opening and mitochondrial membrane depolarization. As a result, mitochondrial respiration is inhibited with less ATP production. The insufficient energy production also arises from the switch from an aerobic metabolism to a high glycolytic metabolic profile. Protein kinase D (PDK), which inhibits pyruvate dehydrogenase during glucose oxidation, is a key factor in the deficient energy supply [25, 26]. Another aspect of redox imbalance is the extracellular matrix (ECM) modification deriving from the matrix metalloproteinases (MMP) activation, which leads to heart remodelling and fibrosis [27].

Within the complex architecture of the heart, the right ventricle (RV) seems to be the most susceptible structure to be affected by the above-mentioned hypoxia-induced changes. The different morphology and metabolism between the left and the right ventricle can in part explain the different susceptibility [28]. Furthermore, the anatomy of most of the CHD exposes the right ventricle to higher stresses, making it more prone to fail than the left counterpart [24]. One of the main insults to which the RV is subjected is the pressure overload that can derive from pulmonary artery hypertension (PAH) or RV outflow obstruction, with both events leading to right ventricular hypertrophy (RVH) and eventually to right ventricular failure.

2.3. HIF-1alpha mediated angiogenic response

One of the key features of chronic hypoxia is the activation of the HIF-1alpha (HIF-1α) signalling, an essential regulator of the angiogenic response. The mechanisms by which HIF-1α is triggered are relatively well-understood: under hypoxia, HIF1-alpha degradation is prevented by the hydroxylation of specific protein residues, and therefore, its translocation to the nucleus promotes the transcriptions of pro-angiogenic genes like vascular endothelial growth factor (VEGF), platelet-derived endothelial cell growth factor/thymidine phosphorylase (PD-ECGF/TP) and erythropoietin (EPO) [29–31].

The role of HIF-1α in adult ischemic heart disease and pressure overload heart failure has been widely demonstrated by different research groups [6, 30, 32]. However, only few studies have investigated its involvement in the pathogenesis of congenital heart disease [6, 33, 34].

An important increase in HIF-1α and related pro-angiogenic genes and proteins have been reported in ventricular biopsies from children with cyanotic congenital heart disease, compared with acyanotic or control groups [22, 35]. In addition, mRNA level of HIF-1α as well as that of two of its representative target genes, VEGF and EPO, were found to be upregulated in blood samples of newborns with cyanosis and persistent pulmonary hypertension, therefore representing early markers of generalized hypoxia [36]. If the HIF-1α/VEGF-induced collateral vessel formation in hypoxemic myocardium is essential to compensate the lack of oxygen supply in cyanotic hearts, especially in cases of coarctation of aorta, an abnormal vessel formation can become a source of morbidity, due to arteriovenous malformations [34]. However, a correlation between VEGF increase and abnormal vessel formation has not yet been found [37]. Nevertheless, increased activation of HIF-1α/VEGF signalling might be detrimental in newborn with persistent pulmonary hypertension, as these patients normally present an overexpression of VEGF receptor 1 (VEGFR1), which accounts for the vasoconstrictor effect of VEGF [38].

Further mechanisms, independent from HIF-1α might account for the hypoxia-induced VEGF production in CHDs. As hypoxia is often associated with tissue damage and apoptosis, cytokines or other mediators (IL-10, TNF-α, TGF-β, etc.) might as well initiate the cascade that leads to VEGF production [31].

2.4. Hypoxia-induced DNA damage

The induction of p53 pathway, as a result of the ROS accumulation triggered by hypoxia, is one of the primary event that initiates the apoptotic cascade that occurs in hypoxic states. The activation of p53 leads to an altered expression of the pro-apoptotic gene Bcl-2, which, in turn, causes the DNA damage [39]. It has been shown that the extent of DNA damage depends on the anatomical anomaly and to the grade of cyanosis, with persistent cyanotic patients being more prone to DNA damage. In particular, children with TOF and with septal defects associated with great vessel anomaly displayed a significantly increased DNA damage compared to the ones with isolated septal defects [39, 40]. These data support the evidence that DNA damage can represent a marker of oxidative stress in CHDs as well as the common biochemical modifications and the oxidant status index.

2.5. miRNA involvement in myocardial adaptation to chronic hypoxia

Among the tissue and circulating biomarkers, microRNAs (miRNAs) have emerged as important tools to assess the hypoxic status of a variety of organs. Briefly, miRNAs are small (19–24 nucleotides) non-coding single-stranded RNAs that form complementary pair with specific target mRNAs to negatively regulate these mRNAs' expression via translational repression or degradation [41]. It has been documented that a hypoxic environment can alter the miRNA profile and their regulation of related pathways, especially with regard to apoptosis/proliferation functions [42]. Furthermore, intensive studies in cardiovascular field have shown how the heart pathophysiology is tightly regulated by miRNAs expression and function [43]. Several miRNAs (i.e. miR-208a, miR21, mi-R29) are involved in myocardial development, and their dysregulation has been linked to cardiac remodelling and hypertrophy; miR-145 upregulation was found in smooth muscle cells of vessels from both a murine model and patients with pulmonary arterial hypertension, whereas plasma upregulation of a huge number of miRNAs (miR-1, miR-133a, miT-499, miR-208) has been reported in patients with acute ischemia and, therefore, hypoxic myocardium [44–48]. Experimental studies performed on cardiac cells further validate the finding that miRNAs expression is modulated with hypoxic stimuli: 145 microRNAs were found to be differently expressed in a study conducted on the human cardiac cell cultured under hypoxia compared the normoxia [49]. Among these, miR-146b was shown to play an important role in the adaptation of cardiomyocytes to chronic hypoxia and its inhibition augmented hypoxia-induced cardiomyocyte apoptosis [50].

A wide array of miRNAs have been reported to be dysregulated in children with CHDs, most of which are crucial in RV development and are specifically linked to a particular defect [24]. In addition, the hypoxic state of some CHDs further affects the miRNA profile of the heart. A recent study by Huang and colleagues shed a light on miRNA-184 as a possible player involved in the mechanism leading to cyanotic CHDs [51]. miRNA-184 expression was, in

fact, markedly decreased in myocardial samples from cyanotic CHDs patients, compared to controls and its suppression *in vitro* was also associated with decreased proliferation and induction of apoptosis, through a mechanism that likely involves the activation of Caspase-3 and -9 by the oxidised miRNA-184 [51]. In another study aimed to evaluate the involvement of miRNAs in the hypoxic response of cardiomyocytes, the expression of miR-138 in myocardial samples of cyanotic patients with TOF was almost twofold miR-138 expression in acyanotic group (VSD) patients [52]. This finding suggests that miR-138 might be used to discriminate TOF from other subtypes of CHDs and further supports the evidence that miRNAs can shed a light on the knowledge of the aetiology of different CHDs and be predictive of the clinical outcome/management of these diseases.

3. Reoxygenation and reperfusion injuries

After a hypoxic event or status of the heart, it is crucial to intervene to re-establish a normal oxygen level. In most cases, the intervention involves heart surgery with cardiopulmonary bypass (CPB) and cardioplegic arrest (CA). During such heart surgery, the standard protocol involves the administration of high level of oxygen upon initiation of CPB and before CA. This causes what is commonly referred to as reoxygenation injury [53]. Following the establishment of CPB, the heart is stopped (ischemic period) to carry out the corrective surgery. When the ischemic heart is reperfused at the end of intervention, a reperfusion injury occurs. The severity of this reperfusion injury depends on the severity of the ischemic period and may be linked to delayed post-operative recovery [54].

It has been widely reported that free oxygen radical formation plays an important role in the development of ischemia-reperfusion injury in the heart as well as in various organs. In the reperfused heart, this oxidant formation derives form a series of interacting pathways in cardiac myocytes and endothelial cells, which involve also leukocyte chemotaxis and inflammation. The white blood cells are, in fact, another great source of ROS: when activated by the binding to the hypoxic endothelium, they produce chemotactic substances and oxygen radicals, which are the main responsible for cellular damage [55]. In addition, nitric oxide (NO) production is greatly increased in post-ischemic hearts, thus impairing vascular reactivity [56].

It has been demonstrated that the damage resulting from the reperfusion event is more severe in hypoxic (low oxygen supply), compared to ischemic (low coronary flow) hearts [57]. When comparing the effects of reperfusion, respectively, in ischemic and hypoxic hearts, Samaja et al. found that the myocardial depression, the energy demand, and the associated O_2 free radicals were higher in the hypoxic rat hearts than the ischemic ones. Furthermore, the hearts subjected to chronic hypoxia are even more prone to the reoxygenation injury than the hearts that have experienced acute hypoxic events. The compensatory changes that occur in chronic lack of oxygen may account for the higher predisposition to generate larger amounts of oxygen radicals with the reintroduction of high levels of oxygen [58].

With many CHDs being characterized by a chronic hypoxic status, the subset of cyanotic children is obviously at a higher risk than the acyanotic CHDs population [59]. Clinical studies have shown that, despite similar cross-clamp times during open heart surgery, cyanotic children have worse clinical outcome and more reoxygenation injury, measured by troponin I release, compared with acyanotic children [11]. The major problem arises from the oxygen reintroduction during the cardiopulmonary bypass (CPB), which is a necessary procedure for the surgical management of CHDs [55]. As the chronic hypoxia produces long-term changes in the myocardial metabolism and function, the sudden oxygen reintroduction further exacerbates these effects. The impaired contractility due to hypoxia-induced calcium overload and the loss of high energy phosphates are examples of the pathological events amplified by the reoxygenation [59, 60]. In addition, the depletion of endogenous antioxidants that characterize chronic cyanosis cannot counteract the oxygen radical-mediated injury when oxygen is reintroduced [61]. On the contrary, minimal changes in the antioxidant reserve capacity were reported before and after the CPB in acyanotic infants, suggesting that, in the absence of hypoxia, a small amount of oxygen free radicals are produced [62].

The effect of reoxygenation injury due to CPB in corrective heart surgery in cyanotic children has further been proven by a significant change in the myocardial gene expression profile [21]. In particular, a wide genome expression array study found 32 significantly downregulated and three upregulated genes in cyanotic heart biopsies taken before and after hyperoxic CPB. Among the upregulated genes after reoxygenation, MOSC1, a factor involved in superoxide generation [63], showed a great increase at a mRNA level, thus suggesting its possible involvement in the increase in CPB-induced oxidative stress. On the other hand, the downregulation of the taurine transporter (TAUT) and the consequent depletion of the documented cardioprotective taurine [64] may in part explain some aspects of the myocardial injury, such as the mitochondrial and myofibers dysfunction. In addition, 8-isoprostane, a reliable marker of oxidative stress, was increased after CPB, and this correlated with the downregulation of keys genetic pathways related to myocardial function and to the reduction in antioxidant defenses [21]. It, therefore, appears obvious that the maintenance of endogenous antioxidants during hypoxia is a crucial determinant of tissue recovery on reoxygenation.

It has been suggested that HIF-α might as well stand as target for cardioprotection upon reoxygenation, by inhibiting mitochondrial oxidative metabolism and therefore reducing the generation of ROS under hypoxia-reoxygenation [6].

MicroRNA expression also appears to be affected by the reoxygenation event. In a study by Bolkier et al., the plasma levels of some cardiac-associated miRNA were dramatically increased after surgery of children undergoing open-heart surgery for CHDs. The increase in the selected miRNAs (microRNA-208a, -208b and -499) correlated with higher troponin levels and delayed hospital discharge [65]. This evidence further justifies the use of circulating miRNAs as biomarkers not only for the diagnosis but also for prognosis and prediction of surgical clinical outcome. In addition, through two different approaches—overexpression and inhibition—miRNAs might represent a suitable target to therapeutically treat those defect characterized by an altered expression of their level.

4. Strategies of surgical intervention

In order to reduce the risk associated with reoxygenation injury in children undergoing open-heart surgery, different interventional strategies have been explored. One of the strategies proposed to avoid this injury is the "controlled reoxygenation", achieved by using a partial pressure of oxygen in arterial blood (PaO_2) similar to the patient's preoperative oxygen saturation when starting CPB [66].

Before its adoption in current clinical practices, several experimental studies on animal models have provided the evidence that the biochemical and the functional status of the cyanotic heart are improved by delaying reoxygenation upon cardiopulmonary bypass. Morita and colleagues set up an *in vivo* experimental animal model where immature piglet hearts were subjected to hypoxemia followed by uncontrolled reoxygenation at high oxygen tension (400 mmHg) or controlled oxygenation at ambient tension (40 mmHg) followed by a raising in the tension to 100 mmHg first and 400 mmHg later. The authors found that lipid peroxidation was reduced while antioxidant reserve capacity preserved in the controlled-reoxygenation group, with this outcome correlating with improved ventricular contractility and functional recovery [67]. In addition, using a modified cold blood cardioplegia, enriched with potassium, the calcium influx was limited and the impaired contractility restored upon reoxygenation [66]. Similar results were obtained in another animal study where controlled normoxic reoxygenation showed a better outcome than abrupt oxygen reintroduction at high pressure. Furthermore, the effect of leukodepletion was examined in this study, in order to verify whether the removal of an important source of ROS, the white blood cells, would minimize the reoxygenation injury. The depletion of leukocytes from the blood-reduced oxygen free radical formation and preserved ventricular contractility at similar extent to the one achieved by controlled reoxygenation [55].

The beneficial effect of controlling the rate of re-introduction of molecular oxygen was also evident in adult patients. Lower lipid peroxidation and preserved antioxidant levels were observed in patients receiving normoxic reoxygenation, compared to the hyperoxic ones, although no significant difference between the two groups was found in the cardiac performance after CBP, likely because this was measured at one low time point of the Starling fraction curve [68]. The controlled-reoxygenation procedure has subsequently been adopted in the operations of cyanotic infants undergoing cardiac surgery, obtaining similar results to the ones seen in the acute experimental model [58].

Subsequent studies have further confirmed these findings and stressed the importance of controlled reoxygenation on starting CPB in cyanotic patients. In two randomized controlled trials including cyanotic children receiving CBP, we showed that the reduced myocardial injury in the controlled normoxic group was accompanied by a reduction in cerebral and hepatic injury, assessed by S100 and *α*GT measurement, which are markers of neuron and hepatocytes damage, respectively [69, 70]. In a different study, we have also analysed the effect of the two reoxygenation approaches on the myocardial gene expression profile of cyanotic paediatric patients undergoing corrective heart surgery. Results showed that the controlled reoxygenation reduced the transcriptomic alteration observed following hyperoxic CPB. The most differentially expressed genes, mainly downregulated, were related

to remodelling and metabolic processes, suggesting that the hearts subjected to hyperoxic reoxygenation had lower adaptation and remodelling capacity than the ones with controlled reoxygenation CBP [21].

Another approach of intervention, in the management of CPB, has involved the effect of whole body temperature during the paediatric cardiac surgery.

Although standard CPB procedures have always been conducted by cooling down the body temperature to 28° (hypothermic CPB), in order to reduce the metabolic rate and oxygen consumption, and therefore to protect organs from ischemic injury, recent evidences have demonstrated that normothermic (35°–37°) CPB is associated with lower inflammatory response and organ injury, both in adult and children [71–73].

In addition, we have shown that normothermic CPB in paediatric patients is also associated with reduced oxidative stress, assessed by troponin I and Isoprostane-8 release, compared with hypothermic CPB, while the inflammatory response has similar levels in the two groups [74].

Other researches have also investigated the effect of the temperature of cardioplegia during paediatric CPB. Warm blood cardioplegia, for long time adopted only in adult heart surgery, has proved to be safe and effective compared to standard cold CPB, with even better hydric balance and hemodynamic stability [75]. Once again, the pre-existent hypoxic status affects the biochemical and clinical outcome of the cardioplegic technique used. We have also shown that while for acyanotic patients the cardioplegic technique is not critical, for cyanotic patients, the use of cold blood cardioplegia with terminal warm blood cardioplegic reperfusion ("hot shot") improves the metabolic and functional recovery. The hot shot cardioplegia resulted in higher reperfusion ATP, ATP/ADP and glutamate levels than acyanotic patients, suggesting that this technique is advantageous only in stressed hearts [76]. Furthermore, the study shows that even if the blood cardioplegia is kept at cold temperature, this still offers a higher myocardial protection, compared to the crystalloid cardioplegia, confirming previous experimental and clinical results [77–79].

Besides CPB strategies, a pharmacological approach could be used as an interventional strategy for perioperative cardioprotection of hypoxic hearts. Experimental studies have shown that the selective inhibition of the enzyme phosphodiesterase-5 (PDE-5) can offer myocardial protection in infant hearts by improving myocardial function and reducing infarct size during reperfusion. However, no direct evidence between this protective effect and the hypoxia-induced injury was shown [80].

As for its established role in hypoxia, HIF-1α has also been investigated as a target for hypoxia-induced myocardial injury in reperfusion. By stabilizing its active form, through the compound dimethyloxyglycine (DMOG), a novel HIF-1α stabilizer, Zhang et al. showed that the progression of hypoxia-induced right ventricle remodelling was significantly reduced in a murine model of chronic hypoxia, most likely as a result of the induction of genes related to adaptive processes [81].

Furthermore, as previously mentioned, miRNAs are being extensively investigated as potential therapeutic tools in the management of CHDs. However, despite the fact that the road ahead looks promising and appealing, some obstacles, like the stability, the off-target effects

and the immunogenicity of the delivery vehicles, still need to be overcome before getting miRNA-based therapeutics into clinical practice.

5. Conclusion

In conclusion, important steps ahead have been made in the knowledge of the mechanisms by which hypoxia takes part to the onset of congenital heart diseases, especially with regard to cyanotic patients. Likewise, significant advances have been made in the strategies of intervention involving open-heart surgery of children with these defects; in order to reduce the injury induced by CPB reoxygenation. Hopefully, the further understanding of the signalling pathways and the mechanism underlying the pathophysiology of hypoxia and hypoxia-induced reoxygenation injury in each kind of defect will result in the development of even better therapeutic strategies and in the design of specific interventions, particularly for the high-risk population.

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References

[1]   Essop MF. Cardiac metabolic adaptations in response to chronic hypoxia. J Physiol. 2007;584(Pt 3):715–26.

[2]   Braunwald E. 50th anniversary historical article. Myocardial oxygen consumption: the quest for its determinants and some clinical fallout. J Am Coll Cardiol. 1999;34(5):1365–8.

[3]   Ostadal B, Ostadalova I, Dhalla NS. Development of cardiac sensitivity to oxygen deficiency: comparative and ontogenetic aspects. Physiol Rev. 1999;79(3):635–59.

[4]   Budev MM, Arroliga AC, Wiedemann HP, Matthay RA. Cor pulmonale: an overview. Semin Respir Crit Care Med. 2003;24(3):233–44.

[5]   Grifka RG. Cyanotic congenital heart disease with increased pulmonary blood flow. Pediatr Clin North Am. 1999;46(2):405–25.

[6]   Semenza GL. Hypoxia-inducible factor 1 and cardiovascular disease. Annu Rev Physiol. 2014;76:39–56.

[7]   Shohet RV, Garcia JA. Keeping the engine primed: HIF factors as key regulators of cardiac metabolism and angiogenesis during ischemia. J Mol Med (Berl). 2007;85(12):1309–15.

[8] Roche SL, Redington AN. The failing right ventricle in congenital heart disease. Can J Cardiol. 2013;29(7):768–78.

[9] Starnes JW, Bowles DK, Seiler KS. Myocardial injury after hypoxia in immature, adult and aged rats. Aging (Milano). 1997;9(4):268–76.

[10] Jonas RA. Myocardial protection for neonates and infants. Thorac Cardiovasc Surg. 1998;46(Suppl. 2):288–91.

[11] Imura H, Caputo M, Parry A, Pawade A, Angelini GD, Suleiman MS. Age-dependent and hypoxia-related differences in myocardial protection during pediatric open heart surgery. Circulation. 2001;103(11):1551–6.

[12] Lopaschuk GD, Spafford MA. Differences in myocardial ischemic tolerance between 1- and 7-day-old rabbits. Can J Physiol Pharmacol. 1992;70(10):1315–23.

[13] Houyel L, Khoshnood B, Anderson RH, Lelong N, Thieulin AC, Goffinet F, et al. Population-based evaluation of a suggested anatomic and clinical classification of congenital heart defects based on the International Paediatric and Congenital Cardiac Code. Orphanet J Rare Dis. 2011;6:64.

[14] Fraser CD, Carberry KE. Congenital heart disease. In: Townsend CM Jr, Beauchamp RD, Evers BM, Mattox KL, eds. *Sabiston Textbook of Surgery*. 19th ed. Philadelphia, PA: Elsevier Saunders; 2012:chap 59.

[15] Webb GD, Smallhorn JF, Therrien J, Redington AN. Congenital heart disease. In: Mann DL, Zipes DP, Libby P, Bonow RO, *Braunwald E, eds. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine*. 10th ed. Philadelphia, PA: Elsevier Saunders; 2015:chap 62.

[16] Rokicki W, Strzalkowski A, Klapcinska B, Danch A, Sobczak A. Antioxidant status in newborns and infants suffering from congenital heart defects. Wiad Lek. 2003;56(7–8):337–40.

[17] Ercan S, Cakmak A, Kosecik M, Erel O. The oxidative state of children with cyanotic and acyanotic congenital heart disease. Anadolu Kardiyol Derg. 2009;9(6):486–90.

[18] Scandalios JG. The rise of ROS. Trends Biochem Sci. 2002;27(9):483–6.

[19] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9–19.

[20] Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sanchez-Perez P, Cadenas S, et al. Antioxidant responses and cellular adjustments to oxidative stress. Redox Biol. 2015;6:183–97.

[21] Ghorbel MT, Mokhtari A, Sheikh M, Angelini GD, Caputo M. Controlled reoxygenation cardiopulmonary bypass is associated with reduced transcriptomic changes in cyanotic tetralogy of Fallot patients undergoing surgery. Physiol Genomics. 2012;44(22):1098–106.

[22] Ghorbel MT, Cherif M, Jenkins E, Mokhtari A, Kenny D, Angelini GD, et al. Transcriptomic analysis of patients with tetralogy of Fallot reveals the effect of chronic hypoxia on myocardial gene expression. J Thorac Cardiovasc Surg. 2010;140(2):337–45.e26.

[23] Sharma K, Kass DA. Heart failure with preserved ejection fraction: mechanisms, clinical features, and therapies. Circ Res. 2014;115(1):79–96.

[24] Iacobazzi D, Suleiman MS, Ghorbel M, George SJ, Caputo M, Tulloh RM. Cellular and molecular basis of RV hypertrophy in congenital heart disease. Heart. 2016;102(1):12–7.

[25] Piao L, Sidhu VK, Fang YH, Ryan JJ, Parikh KS, Hong Z, et al. FOXO1-mediated upregulation of pyruvate dehydrogenase kinase-4 (PDK4) decreases glucose oxidation and impairs right ventricular function in pulmonary hypertension: therapeutic benefits of dichloroacetate. J Mol Med (Berl). 2013;91(3):333–46.

[26] Ryan JJ, Archer SL. The right ventricle in pulmonary arterial hypertension: disorders of metabolism, angiogenesis and adrenergic signaling in right ventricular failure. Circ Res. 2014;115(1):176–88.

[27] Qipshidze N, Tyagi N, Metreveli N, Lominadze D, Tyagi SC. Autophagy mechanism of right ventricular remodeling in murine model of pulmonary artery constriction. Am J Physiol Heart Circ Physiol. 2012;302(3):H688–96.

[28] Friedberg MK, Redington AN. Right versus left ventricular failure: differences, similarities, and interactions. Circulation. 2014;129(9):1033–44.

[29] Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science. 2001;292(5516):468–72.

[30] Semenza GL. O2-regulated gene expression: transcriptional control of cardiorespiratory physiology by HIF-1. J Appl Physiol (1985). 2004;96(3):1173–7; discussion 0-2.

[31] Felmeden DC, Blann AD, Lip GY. Angiogenesis: basic pathophysiology and implications for disease. Eur Heart J. 2003;24(7):586–603.

[32] Holscher M, Silter M, Krull S, von Ahlen M, Hesse A, Schwartz P, et al. Cardiomyocyte-specific prolyl-4-hydroxylase domain 2 knock out protects from acute myocardial ischemic injury. J Biol Chem. 2011;286(13):11185–94.

[33] Himeno W, Akagi T, Furui J, Maeno Y, Ishii M, Kosai K, et al. Increased angiogenic growth factor in cyanotic congenital heart disease. Pediatr Cardiol. 2003;24(2):127–32.

[34] El-Melegy NT, Mohamed NA. Angiogenic biomarkers in children with congenital heart disease: possible implications. Ital J Pediatr. 2010;36:32.

[35] Qing M, Gorlach A, Schumacher K, Woltje M, Vazquez-Jimenez JF, Hess J, et al. The hypoxia-inducible factor HIF-1 promotes intramyocardial expression of VEGF in infants with congenital cardiac defects. Basic Res Cardiol. 2007;102(3):224–32.

[36] Lemus-Varela ML, Flores-Soto ME, Cervantes-Munguia R, Torres-Mendoza BM, Gudino-Cabrera G, Chaparro-Huerta V, et al. Expression of HIF-1 alpha, VEGF and EPO in peripheral blood from patients with two cardiac abnormalities associated with hypoxia. Clin Biochem. 2010;43(3):234–9.

[37] Ootaki Y, Yamaguchi M, Yoshimura N, Oka S, Yoshida M, Hasegawa T. Vascular endothelial growth factor in children with congenital heart disease. Ann Thorac Surg. 2003;75(5):1523–6.

[38] Nadeau S, Baribeau J, Janvier A, Perreault T. Changes in expression of vascular endothelial growth factor and its receptors in neonatal hypoxia-induced pulmonary hypertension. Pediatr Res. 2005;58(2):199–205.

[39] Vidya G, Suma HY, Vishnu Bhat B, Parkash Chand, Ramachandra Rao K. Hypoxia induced DNA damage in children with isolated septal defect and septal defect with great vessel anomaly of heart. J Clin Diagn Res. 2014;8(4):SC01–3.

[40] Srujana K, Begum SS, Rao KN, Devi GS, Jyothy A, Prasad MH. Application of the comet assay for assessment of oxidative DNA damage in circulating lymphocytes of Tetralogy of Fallot patients. Mutat Res. 2010;688(1–2):62–5.

[41] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281–97.

[42] Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ, et al. A microRNA signature of hypoxia. Mol Cell Biol. 2007;27(5):1859–67.

[43] Condorelli G, Latronico MV, Cavarretta E. microRNAs in cardiovascular diseases: current knowledge and the road ahead. J Am Coll Cardiol. 2014;63(21):2177–87.

[44] Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature. 2008;456(7224):980–4.

[45] van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci U S A. 2008;105(35):13027–32.

[46] Caruso P, Dempsie Y, Stevens HC, McDonald RA, Long L, Lu R, et al. A role for miR-145 in pulmonary arterial hypertension: evidence from mouse models and patient samples. Circ Res. 2012;111(3):290–300.

[47] Wang GK, Zhu JQ, Zhang JT, Li Q, Li Y, He J, et al. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. Eur Heart J. 2010;31(6):659–66.

[48] Cheng Y, Tan N, Yang J, Liu X, Cao X, He P, et al. A translational study of circulating cell-free microRNA-1 in acute myocardial infarction. Clin Sci (Lond). 2010;119(2):87–95.

[49] Chen YT, Liew OW, Richards AM. MicroRNA expression profiles of human left ventricle derived cardiac cells in normoxic and hypoxic conditions. Genom Data. 2015;5:59–60.

[50] Li JW, He SY, Feng ZZ, Zhao L, Jia WK, Liu P, et al. MicroRNA-146b inhibition augments hypoxia-induced cardiomyocyte apoptosis. Mol Med Rep. 2015;12(5):6903–10.

[51] Huang J, Li X, Li H, Su Z, Wang J, Zhang H. Down-regulation of microRNA-184 contributes to the development of cyanotic congenital heart diseases. Int J Clin Exp Pathol. 2015;8(11):14221–7.

[52] He S, Liu P, Jian Z, Li J, Zhu Y, Feng Z, et al. miR-138 protects cardiomyocytes from hypoxia-induced apoptosis via MLK3/JNK/c-jun pathway. Biochem Biophys Res Commun. 2013;441(4):763–9.

[53] Tan S, Yokoyama Y, Wang Z, Zhou F, Nielsen V, Murdoch AD, et al. Hypoxia-reoxygenation is as damaging as ischemia-reperfusion in the rat liver. Crit Care Med. 1998;26(6):1089–95.

[54] Ferrari R, Alfieri O, Curello S, Ceconi C, Cargnoni A, Marzollo P, et al. Occurrence of oxidative stress during reperfusion of the human heart. Circulation. 1990;81(1):201–11.

[55] Allen BS, Ilbawi MN. Hypoxia, reoxygenation and the role of systemic leukodepletion in pediatric heart surgery. Perfusion. 2001;16(Suppl):19–29.

[56] Zweier JL, Talukder MA. The role of oxidants and free radicals in reperfusion injury. Cardiovasc Res. 2006;70(2):181–90.

[57] Samaja M, Motterlini R, Santoro F, Dell' Antonio G, Corno A. Oxidative injury in reoxygenated and reperfused hearts. Free Radic Biol Med. 1994;16(2):255–62.

[58] Allen BS, Rahman S, Ilbawi MN, Kronon M, Bolling KS, Halldorsson AO, et al. Detrimental effects of cardiopulmonary bypass in cyanotic infants: preventing the reoxygenation injury. Ann Thorac Surg. 1997;64(5):1381–7; discussion 7-8.

[59] Corno AF, Milano G, Samaja M, Tozzi P, von Segesser LK. Chronic hypoxia: a model for cyanotic congenital heart defects. J Thorac Cardiovasc Surg. 2002;124(1):105–12.

[60] Najm HK, Wallen WJ, Belanger MP, Williams WG, Coles JG, Van Arsdell GS, et al. Does the degree of cyanosis affect myocardial adenosine triphosphate levels and function in children undergoing surgical procedures for congenital heart disease? J Thorac Cardiovasc Surg. 2000;119(3):515–24.

[61] Teoh KH, Mickle DA, Weisel RD, Li RK, Tumiati LC, Coles JG, et al. Effect of oxygen tension and cardiovascular operations on the myocardial antioxidant enzyme activities in patients with tetralogy of Fallot and aorta-coronary bypass. J Thorac Cardiovasc Surg. 1992;104(1):159–64.

[62] Bolling KS, Halldorsson A, Allen BS, Rahman S, Wang T, Kronon M, et al. Prevention of the hypoxic reoxygenation injury with the use of a leukocyte-depleting filter. J Thorac Cardiovasc Surg. 1997;113(6):1081–9; discussion 9-90.

[63] Morita K, Ihnken K, Buckberg GD, Sherman MP, Young HH. Studies of hypoxemic/reoxygenation injury: without aortic clamping. IX. Importance of avoiding perioperative hyperoxemia in the setting of previous cyanosis. J Thorac Cardiovasc Surg. 1995;110(4 Pt 2):1235–44.

[64] Ito T, Kimura Y, Uozumi Y, Takai M, Muraoka S, Matsuda T, et al. Taurine depletion caused by knocking out the taurine transporter gene leads to cardiomyopathy with cardiac atrophy. J Mol Cell Cardiol. 2008;44(5):927–37.

[65] Bolkier Y, Nevo-Caspi Y, Salem Y, Vardi A, Mishali D, Paret G. Micro-RNA-208a, -208b, and -499 as biomarkers for myocardial damage after cardiac surgery in children. Pediatr Crit Care Med. 2016;17(4):e193–7.

[66] Ihnken K, Morita K, Buckberg GD. Delayed cardioplegic reoxygenation reduces reoxygenation injury in cyanotic immature hearts. Ann Thorac Surg. 1998;66(1):177–82.

[67] Morita K, Ihnken K, Buckberg GD. Studies of hypoxemic/reoxygenation injury: with aortic clamping. XII. Delay of cardiac reoxygenation damage in the presence of cyanosis: a new concept of controlled cardiac reoxygenation. J Thorac Cardiovasc Surg. 1995;110(4 Pt 2):1265–73.

[68] Ihnken K, Winkler A, Schlensak C, Sarai K, Neidhart G, Unkelbach U, et al. Normoxic cardiopulmonary bypass reduces oxidative myocardial damage and nitric oxide during cardiac operations in the adult. J Thorac Cardiovasc Surg. 1998;116(2):327–34.

[69] Caputo M, Mokhtari A, Rogers CA, Panayiotou N, Chen Q, Ghorbel MT, et al. The effects of normoxic versus hyperoxic cardiopulmonary bypass on oxidative stress and inflammatory response in cyanotic pediatric patients undergoing open cardiac surgery: a randomized controlled trial. J Thorac Cardiovasc Surg. 2009;138(1):206–14.

[70] Caputo M, Mokhtari A, Miceli A, Ghorbel MT, Angelini GD, Parry AJ, et al. Controlled reoxygenation during cardiopulmonary bypass decreases markers of organ damage, inflammation, and oxidative stress in single-ventricle patients undergoing pediatric heart surgery. J Thorac Cardiovasc Surg. 2014;148(3):792–801, e8; discussion 0-1.

[71] Birdi I, Caputo M, Underwood M, Angelini GD, Bryan AJ. Influence of normothermic systemic perfusion temperature on cold myocardial protection during coronary artery bypass surgery. Cardiovasc Surg. 1999;7(3):369–74.

[72] Birdi I, Regragui I, Izzat MB, Bryan AJ, Angelini GD. Influence of normothermic systemic perfusion during coronary artery bypass operations: a randomized prospective study. J Thorac Cardiovasc Surg. 1997;114(3):475–81.

[73] Ohata T, Sawa Y, Kadoba K, Taniguchi K, Ichikawa H, Masai T, et al. Normothermia has beneficial effects in cardiopulmonary bypass attenuating inflammatory reactions. ASAIO J. 1995;41(3):M288–91.

[74] Caputo M, Bays S, Rogers CA, Pawade A, Parry AJ, Suleiman S, et al. Randomized comparison between normothermic and hypothermic cardiopulmonary bypass in pediatric open-heart surgery. Ann Thorac Surg. 2005;80(3):982–8.

[75] Durandy Y, Hulin S. Intermittent warm blood cardioplegia in the surgical treatment of congenital heart disease: clinical experience with 1400 cases. J Thorac Cardiovasc Surg. 2007;133(1):241–6.

[76] Modi P, Suleiman MS, Reeves B, Pawade A, Parry AJ, Angelini GD, et al. Myocardial metabolic changes during pediatric cardiac surgery: a randomized study of 3 cardioplegic techniques. J Thorac Cardiovasc Surg. 2004;128(1):67–75.

[77] Bolling K, Kronon M, Allen BS, Wang T, Ramon S, Feinberg H. Myocardial protection in normal and hypoxically stressed neonatal hearts: the superiority of blood versus crystalloid cardioplegia. J Thorac Cardiovasc Surg. 1997;113(6):994–1003; discussion 1003-5.

[78] del Nido PJ, Mickle DA, Wilson GJ, Benson LN, Weisel RD, Coles JG, et al. Inadequate myocardial protection with cold cardioplegic arrest during repair of tetralogy of Fallot. J Thorac Cardiovasc Surg. 1988;95(2):223–9.

[79] Modi P, Imura H, Caputo M, Pawade A, Parry A, Angelini GD, et al. Cardiopulmonary bypass-induced myocardial reoxygenation injury in pediatric patients with cyanosis. J Thorac Cardiovasc Surg. 2002;124(5):1035–6.

[80] Bremer YA, Salloum F, Ockaili R, Chou E, Moskowitz WB, Kukreja RC. Sildenafil citrate (viagra) induces cardioprotective effects after ischemia/reperfusion injury in infant rabbits. Pediatr Res. 2005;57(1):22–7.

[81] Zhang S, Ma K, Liu Y, Pan X, Chen Q, Qi L, et al. Stabilization of hypoxia-inducible factor by DMOG inhibits development of chronic hypoxia-induced right ventricular remodeling. J Cardiovasc Pharmacol. 2016;67(1):68–75.