Published in final edited form as:

Pediatr Cardiol. 2011 April ; 32(4): . doi:10.1007/s00246-010-9870-y.

Histopathologic Evaluation of Patent Ductus Arteriosus Stents After Hybrid Stage I Palliation

Matthew J. Egan[#],

The Heart Center, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH 43205, USA

Aaron J. Trask[#],

The Heart Center, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH 43205, USA

Peter B. Baker,

Department of Pathology, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH 43205, USA

Jonathan Lawrence,

The Heart Center, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH 43205, USA

Elena Ladich,

CVPPath Institute, Gaithersburg, MD, USA

Renu Virmani,

CVPPath Institute, Gaithersburg, MD, USA

Sharon L. Hill,

The Heart Center, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH 43205, USA

John P. Cheatham,

The Heart Center, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH 43205, USA

Mark Galantowicz,

The Heart Center, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH 43205, USA

Pamela A. Lucchesi, and

The Heart Center, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH 43205, USA

John P. Kovalchin

The Heart Center, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH 43205, USA

[#] These authors contributed equally to this work.

Abstract

The aim of this study was to determine the histopathology of patent ductus arteriosus (PDA) in-stent stenosis after hybrid stage I palliation. The hybrid approach to palliation of hypoplastic left heart syndrome can be complicated by the development of in-stent stenosis of the PDA. This may obstruct retrograde aortic arch flow, decrease systemic circulation, and lead to interstage interventional procedures. Stented PDA samples removed from eight patients undergoing comprehensive stage II repair were examined by way of radiography and histochemistry (hematoxylin and eosin, Movat pentachrome, α -smooth muscle actin, and proliferating cell nuclear antigen). A retrospective chart review of the patients was also performed. PDA stents were in place in the PDA for a mean period of 169 ± 28 days in patients who had a mean age of 176 ± 30 days at the time of stent removal. Stent deployment caused chronic inflammation, caused fibrin deposition, and induced vascular smooth muscle-cell (VSMC) proliferation in the area immediately surrounding the stent struts. The neointimal region was composed largely of smooth muscle cells that appeared to be fully differentiated by the lack of PCNA staining. Neointimal thickening occurs in the PDA after stent placement for hybrid palliation of HLHS and is the result of inflammation, extracellular matrix deposition, and smooth muscle-cell proliferation in the peristrut region. This finding suggests that proliferating VSMCs in the peristrut region may provide the impetus for inward neointimal formation and therefore the manifestation of in-stent stenosis.

Keywords

Ductus arteriosus; Stent; Neointima; In-stent stenosis

Hypoplastic left heart syndrome (HLHS) is a congenital condition whereby the left ventricle and aorta are under-developed, resulting in a lethal impairment in cardiac output if left untreated. Patients with HLHS undergo a series of palliative procedures, beginning in the neonatal period, to separate the systemic and pulmonary circulations. The hybrid approach to palliation of HLHS utilizing pulmonary artery bands, stent placement in the patent ductus arteriosus (PDA), and atrial septostomy, has emerged as an alternative treatment strategy [6, 7]. One complication in these patients is the development of PDA in-stent stenosis. Only a few studies have demonstrated that in-stent stenosis occurs in the ductus arteriosus with increasing frequency the longer the stent remains in place [2, 8].

The obstruction of blood flow through the PDA stent can cause multiple hemodynamic sequelae. In patients with HLHS, this may cause decreased systemic perfusion, decreased retrograde perfusion of the head and neck vessels, and increased afterload on the right ventricle. Patients are followed up closely during the interstage time period before the comprehensive stage II repair (bidirectional cavopulmonary anastomosis, removal of the pulmonary artery bands and PDA stent, Damus-Kaye-Stansel procedure, aortic arch reconstruction, and atrial septectomy). Patients undergo serial echocardiograms to monitor for increases in blood flow velocity through the PDA stent, which indicates blood flow obstruction.

Neointimal formation in coronary stents has been previously described, and these studies led to the development of drug-eluting stents in an effort to decrease neointimal formation [5, 11]. The composition of tissue in the ductus arteriosus differs from coronary artery tissue because it is programmed to close spontaneously through a complex process involving both vasoconstriction and neointima cushion formation [1]. The purpose of this study was to determine the histopathologic basis of tissue growth in PDA stents in patients with HLHS undergoing hybrid stage I palliation, which has not been previously examined.

Methods

Patient charts were evaluated for the underlying diagnosis of HLHS, the age of the patient at the time of stent removal, and the duration that the PDA stent was in place. If a patient had previously undergone intervention for in-stent stenosis, the timing of this procedure was noted.

Eight consecutive patients with single-ventricle anatomy who had undergone hybrid stage I palliation and who had ductal stent fragments and associated tissue available for analysis were included in the study. In our overall patient population, approximately 75% of the patients underwent placement of a self-expandable stent, and 25% of the patients underwent placement of a balloon-expandable stent. The self-expandable stent allows the contour of the PDA and junction of the aorta, as well as the differing diameters, to be readily accommodated. Balloon-expandable stents, however, are typically placed if there is preexisting PDA stenosis or if the length of the PDA would be inappropriate for a 20 mm-long self-expandable stent.

The size and length of the PDA stent to be implanted are based on several factors. Through the short sheath that is placed by the surgeon by way of purse-string sutures at the sinotubular junction of the pulmonary valve and pulmonary artery, a V-18 guidewire is passed under direct fluoroscopic examination from a straight lateral view, down the ductus arteriosus, and into the descending aorta. The angiogram is then performed through the sidearm of the sheath with a 20-mm calibration sphere, which is used for precise measurement. The PDA diameter is measured in end systole and at its largest diameter at the pulmonary entrance of the PDA, which is typically at the junction of the left pulmonary artery and main pulmonary artery-PDA, at the mid-portion of the PDA, and at the aortic transition zone. The length of the PDA is then determined to ensure covering the entire length of the PDA tissue, which necessitates it being proximal to the junction of the left pulmonary artery and PDA, and extending the entire length of the PDA. Frequently, the stent extends distal to the retrograde aortic arch origin and into the descending aorta where ductal tissue is usually found. Typically, the diameter of the stent is then chosen to be approximately 1 mm larger than the diameter of the PDA at the level of the pulmonary entrance, and it is frequently slightly smaller than at the mid-portion of the PDA, which is the largest diameter, and 2 mm larger than the diameter at the aortic transition zone. Regardless of the size of the patient, the PDA diameters are relatively consistent.

In this series, all eight patients had self-expanding nitinol stents placed in the ductus arteriosus after angiographic measurements of the ductus arteriosus were made during hybrid stage I palliation. Stented PDA tissue was removed from patients at the time of comprehensive stage II repair. The stent tissue was fixed in 4% paraformaldehyde for 4 hours and stored in 15% sucrose until embedding. The stented vessel segments were then dehydrated in a graded series of ethanol and embedded in methylmethacrylate plastic. After polymerization, sections from the stents were cut on a rotary microtome at 6 μ m, mounted, and stained with hematoxylin and eosin, and Movat pentachrome stains. All sections were examined by light microscopy for the presence of inflammation, thrombus, and vessel-wall injury. For sections stained with α -smooth muscle actin (α -SMA) and proliferating cell nuclear antigen (PCNA; Dako, Carpinteria, CA), the tissue was pretreated with antigen retrieval/ethylene diamine tetraacetic acid (EDTA) for 10 min and then incubated with the primary antibody overnight at 2°C to 8°C. This was followed by a labeled streptavidin biotin (LSAB) link (Dako) and LSAB label (Dako) at 30 minutes each and chromogen development (3-amino-9-ethylcarbazole) for 5 to 10 min at room temperature. All study procedures were approved by our Institutional Review Board.

Results

The patient population included six patients with a classic form of HLHS, while 2 patients had more complex forms of single-ventricle anatomy who also underwent hybrid stage I palliation (Table 1). In this patient population, the stents ranged from 7 to 9 mm in diameter and were 20 mm long. Patients had a mean age of 176 ± 30 days at the time of stent removal during the comprehensive stage II procedure, and the PDA stents were in place for a mean of 169 ± 28 days at the time of removal. One patient had an additional PDA stent placed inside the previous stent to treat the obstruction at 3 months and 18 days of age. A second patient was referred for earlier comprehensive stage II repair at 4 months and 13 days of age due to significant in-stent stenosis.

Histological evaluation of the stented PDA sections showed the presence of a thickened neointima composed of proteoglycans and smooth muscle cells (Fig. 1). Stent deployment caused chronic inflammation, fibrin deposition, and induced vascular smooth muscle cell (VSMC) proliferation in the area immediately surrounding the stent struts (Fig. 1 [inset]).

-SMA staining showed the presence of VSMCs throughout the section, including in the neointima and peristrut region of the stent (Fig. 2 [left]). Surprisingly, PCNA staining was not present in the neointimal region but was highly expressed in the peristrut region where VSMCs were densely populated (Fig. 2 [right]). The neointimal region was composed largely of smooth muscle cells that appeared to be fully differentiated demonstrated by the lack of PCNA staining.

Discussion

The current study is the first histological examination of stent deployment in the PDA during hybrid stage I palliation of patients with single-ventricle physiology and demonstrates robust proliferation of VSMCs in the region immediately surrounding the stent strut. The proliferation marker PCNA and the VSMC marker α -SMA were highly expressed in the peristrut region of the nitinol stents, and Movat's pentachrome stain showed the presence of chronic inflammation and extracellular matrix (fibrin), and proteoglycan deposition in the peristrut region of the stent. Compared with the peristrut region, the neointima was largely devoid of PCNA staining but rich in VSMCs, suggesting that VSMCs in the neointima of the stented PDA were fully differentiated.

Various options for palliative procedures for ductal-dependent congenital heart disease, such as HLHS, involve the placement of PDA stents. The morphology of the ductus arteriosus in patients with HLHS is typically widely patent, which can differ from more tortuous PDAs, that can be found in patients with other forms of ductal-dependent congenital heart disease. One potential complication, however, is the development of in-stent stenosis in some patients secondary to an unknown mechanism. Since Coe et al. [4] first described that stenting the ductus arteriosus in a newborn lamb model maintains its patency for at least 3 months, few advances in the mechanisms of in-stent stenosis have been made. An early study in a piglet model demonstrated that balloon-expandable stents inserted into the ductus arteriosus resulted in high variability in the patency of blood flow through this tissue with several PDA stents maintaining patency for at least 30 days [9]. This variability in stented ductus arteriosus neointimal formation, leading to significant in-stent stenosis or retrograde aortic arch obstruction, is also evident in patients after hybrid stage I palliation [16]. Other investigators have reported a wide range of 25% to 100% luminal narrowing, which occurs as early as 4 months in patients with ductal-dependent congenital heart disease [14]. A report by Rosenthal et al. [13] showed that the ductus arteriosus in the lamb remained patent for 16 to 21 months after stent deployment into the vessel, although there was significant

neointima present at 6 months of age. Although blood flow through the stent was not measured, it remained unclear whether the in-stent stenosis was flow limiting. Our current data suggest that in-stent stenosis occurs as a result of the formation of a neointima composed of differentiated smooth muscle cells that progresses inward as a result of smooth muscle–cell proliferation in the peristrut region of the stent.

Although little is known about in-stent stenosis in PDA tissue, in-stent restenosis and neointimal formation have been the focus of much scientific study in the coronary vasculature. It is well documented and widely agreed on that coronary restenosis occurs primarily due to the formation of neointimal tissue consisting of smooth muscle cells and proteoglycan matrix; however, the mechanisms behind the restenosis remain unclear. Restenosis seems to occur in sequential stages and includes thrombus formation in the peristrut region of the stent, local inflammation, and deep arterial injury [17]. In stented porcine coronary arteries, cellular proliferation was greatest at 7 days and returned to basal levels 28 days after left main coronary stent deployment, a time when neointimal thickness was highest compared with 1, 7, and 14 days after stent placement [3]. Similarly, Nakatani et al. [12] reported scarce PCNA staining in coronary artery neointima 28 days after stent deployment in the left anterior descending coronary artery, indicating the lack of proliferation in the neointimal region. In keeping with these findings, our current data indicate that proliferation of ductal VSMCs in the neointimal region was relatively low at an average of 6 months after stent implantation but that VSMC proliferation and inflammation was higher in the area immediately surrounding the stent struts.

Although these potential factors influencing coronary restenosis may contribute to in-stent restenosis, it is not known if the same mechanisms can be applied to the PDA tissue because this unique vascular tissue normally contracts shortly after birth. Several studies may provide insight. Per the criteria of coronary restenosis by Virmani and Farb, our current data show that local inflammation surrounding the peristrut region of the stents may be a causative factor for vascular injury, thereby providing the impetus for VSMC proliferation in the same region [17]. The presence of proliferating VSMCs in the peristrut region of the PDA suggests that this area of damage may induce active proliferation of VSMCs that intrude into the lumen, pushing the neointima inward, thus chronically obstructing blood flow. Moreover, the presence of α -SMA staining in the neointima and the lack of PCNA staining in the same region suggests that the PDA VSMCs are differentiated. Advanced differentiation and augmented contractile components of VSMCs are a normal phenotype in the ductus arteriosus in maturing fetuses and during the neonatal period, but the impact of this VSMC phenotype in PDA tissue on in-stent restenosis is unknown [15]. Furthermore, one study demonstrated the importance of the T-type voltage-dependent calcium channel in the normal closure of the ductus arteriosus in rats [1]. Another recent study demonstrated a decrease of neointimal formation after the implantation of rapamycin-eluting stents in a pig model, suggesting the involvement of the mammalian target of rapamycin in the promotion of neointimal tissue [10]. These molecular targets may provide a rationale for the use of drug-eluting stents in ductal-dependent congenital heart diseases; however, it remains to be determined whether drug-eluting stents should be used clinically because they have been shown to impair neointimal healing and promote thrombus formation. Significant clinical investigation remains to demonstrate the feasibility and safety of drug-eluting stents in infants.

Limitations

A limitation of this study is that in this patient population, there was only tissue available for analysis from those who underwent placement of a self-expanding stent in the ductus arteriosus. Also, due to the limited amount of tissue available from some patients, a

comparative analysis between the patients who required repeat PDA stent placement and those who did not was not performed.

Conclusion

The clinical implications of maintaining patency of the ductus arteriosus during hybrid stage I palliation of HLHS are paramount. Significant neointimal formation can lead to hemodynamic alterations in patients after initial palliation and can lead to additional interstage interventional procedures. This histologic evaluation of in-stent stenosis suggests that proliferating VSMCs in the peristrent region may provide the impetus for inward neointimal formation and thus the manifestation of in-stent stenosis. Furthermore, it serves as a continual basis for further investigation to better understand the mechanisms behind in-stent stenosis in this unique vascular tissue and attempt to find ways to minimize its occurrence.

Acknowledgments

This work was supported in part by the National Institute of Health [LBI 2RO1HL5604-12 (PAL), 5T32HL098039 (AJT, PAL)] and The Heart Center at Nationwide Children's Hospital. The authors also gratefully acknowledge the assistance of Naima Carter-Monroe, M.D. from CVPPath Institute, Inc. in Gaithersburg, Maryland. The studies outlined in this manuscript were supported by The Center for Cardiovascular and Pulmonary Research and The Heart Center at Nationwide Children's Hospital, Columbus, OH.

References

1. Akaike T, Jin MH, Yokoyama U, Izumi-Nakaseko H, Jiao Q, Iwasaki S, et al. T-type Ca²⁺ channels promote oxygenation-induced closure of the rat ductus arteriosus not only by vasoconstriction but also by neointima formation. *J Biol Chem*. 2009; 284:24025–24034. [PubMed: 19567868]
2. Alwi M, Choo KK, Latiff HA, Kandavello G, Samion H, Mulyadi MD. Initial results and medium-term follow-up of stent implantation of patent ductus arteriosus in duct-dependent pulmonary circulation. *J Am Coll Cardiol*. 2004; 44:438–445. [PubMed: 15261945]
3. Carter AJ, Laird JR, Farb A, Kufs W, Wortham DC, Virmani R. Morphologic characteristics of lesion formation and time course of smooth muscle cell proliferation in a porcine proliferative restenosis model. *J Am Coll Cardiol*. 1994; 24:1398–1405. [PubMed: 7930266]
4. Coe JY, Olley PM. A novel method to maintain ductus arteriosus patency. *J Am Coll Cardiol*. 1991; 18:837–841. [PubMed: 1869747]
5. Costa MA, Simon DI. Molecular basis of restenosis and drug-eluting stents. *Circulation*. 2005; 111:2257–2273. [PubMed: 15867193]
6. Galantowicz M, Cheatham JP. Lessons learned from the development of a new hybrid strategy for the management of hypoplastic left heart syndrome. *Pediatr Cardiol*. 2005; 26:190–199.
7. Galantowicz M, Cheatham JP, Phillips A, Cua CL, Hoffman TM, Hill SL, et al. Hybrid approach for hypoplastic left heart syndrome: intermediate results after the learning curve. *Ann Thorac Surg*. 2008; 85:2063–2070. [PubMed: 18498821]
8. Gibbs JL, Uzun O, Blackburn ME, Wren C, Hamilton JR, Watterson KG. Fate of the stented arterial duct. *Circulation*. 1999; 99:2621–2625. [PubMed: 10338453]
9. Hijazi ZM, Aronovitz MJ, Marx GR, Fulton DR. Successful placement and re-expansion of a new balloon expandable stent to maintain patency of the ductus arteriosus in a newborn animal model. *J Invasive Cardiol*. 1993; 5:351–357. [PubMed: 10146598]
10. Lee KJ, Hinek A, Chaturvedi RR, Almeida CL, Honjo O, Koren G, et al. Rapamycin-eluting stents in the arterial duct: experimental observations in the pig model. *Circulation*. 2009; 119:2078–2085. [PubMed: 19349326]
11. Lowe HC, Schwartz RS, MacNeill BD, Jang IK, Hayase M, Rogers C, et al. The porcine coronary model of in-stent restenosis: current status in the era of drug-eluting stents. *Catheter Cardiovasc Interv*. 2003; 60:515–523. [PubMed: 14624433]

12. Nakatani M, Takeyama Y, Shibata M, Yorozuya M, Suzuki H, Koba S, et al. Mechanisms of restenosis after coronary intervention: difference between plain old balloon angioplasty and stenting. *Cardiovasc Pathol*. 2003; 12:40–48. [PubMed: 12598017]
13. Rosenthal E, Qureshi SA, Tabatabaie AH, Persaud D, Kakadekar AP, Baker EJ, et al. Medium-term results of experimental stent implantation into the ductus arteriosus. *Am Heart J*. 1996; 132:657–663. [PubMed: 8800039]
14. Schneider M, Zartner P, Sidiropoulos A, Konertz W, Hausdorf G. Stent implantation of the arterial duct in newborns with duct-dependent circulation. *Eur Heart J*. 1998; 19:1401–1409. [PubMed: 9792267]
15. Slomp J, Gittenberger-de Groot AC, Glukhova MA, Conny van Munsteren J, Kockx MM, Schwartz SM, et al. Differentiation, dedifferentiation, and apoptosis of smooth muscle cells during the development of the human ductus arteriosus. *Arterioscler Thromb Vasc Biol*. 1997; 17:1003–1009. [PubMed: 9157967]
16. Stoica SC, Phillips AB, Egan M, Rodeman R, Chisolm J, Hill SL, et al. The retrograde aortic arch in the hybrid approach to hypoplastic left heart syndrome. *Ann Thorac Surg*. 2009; 88:1939–1946. [PubMed: 19932266]
17. Virmani R, Farb A. Pathology of in-stent restenosis. *Curr Opin Lipidol*. 1999; 10:499–506. [PubMed: 10680043]

Fig. 1. Representative photomicrographs of Movat's Pentachrome and H&E stained stented ductus arteriosus. Movat's stain revealed neointima that was associated with increased inflammatory and smooth muscle cells in the area immediately surrounding the Nitinol stent struts

Fig. 2. Photomicrographs of PCNA (*left*) showing actively proliferating cells in the area immediately surrounding stent struts. -smooth muscle actin stain (*right*) confirms that the proliferating cells are smooth muscle

Table 1

Underlying diagnosis and anatomy of patients

Anatomy	Patients
HLHS ($n = 6$)	
Aortic atresia/mitral atresia	4
Aortic atresia/mitral stenosis	1
Aortic stenosis/mitral stenosis	1
Complex single ventricle ($n = 2$)	
Double inlet left ventricle	1
Unbalanced AVSD, double outlet right ventricle	1