The Outflow Tract in Transposition of the Great Arteries: an anatomical and morphological study

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Abstract

Objective. Neo-aortic root dilatation is observed after the arterial switch operation for transposition of the great arteries. Although structural differences in the vessel wall of these patients may be of influence, we hypothesize that a histo-morphological difference in composition and embedding of the fibrous annulus in transposition of the great arteries may play a role in neo-aortic root dilatation.

Methods. Two normal human hearts and two unoperated human hearts with transposition of the great arteries, one day postnatal, were studied. Histological sections stained for collagen, myocardium and elastin were prepared and three-dimensional reconstructions of the outflow tracts were made to enable comparison of the morphological structures between the normal hearts and transposition of the great arteries.

Results. The amount of collagen in the arterial roots was diminished in transposition of the great arteries compared to the normal hearts. In addition, also the anchorage and embedding of both arterial roots in the myocardium was less extensive in transposition of the great arteries. The changed position of the arteries in the malformed hearts results in less support for the roots from the surrounding atrioventricular myocardium.

Conclusions. The combination of the observed histo-morphological differences in amount of collagen and myocardial support might be an explanation for the neo-aortic root dilatation observed after the arterial switch operation. The developmental background of the observed deficient fibrous annulus formation might originate from an epicardial problem.
Introduction

Transposition of the great arteries (TGA) is a common and severe congenital heart malformation, having an incidence of about 1 per 5,000 live births. In TGA, the atria and ventricles are in the normal position (situs solitus and concordant atrioventricular connection), but the aorta arises from the right ventricle and the pulmonary trunk arises from the left ventricle (disconcordant ventriculo-arterial connection) changing the serially connected pulmonary and systemic circulations into two parallel (separated) circulations.

The arterial switch operation (ASO) is now the surgical procedure of choice for TGA. This operation involves transsection and reanastomosis of both, pulmonary trunk and aorta, above the sinuses of Valsalva and transplantation of the coronary arteries. After the ASO, the pulmonary root (neo-aortic root) functions in the systemic circulation. The long-term success of this operation depends upon the capability of the native pulmonary root to function in the systemic circulation.

Neo-aortic root dilatation is one of the late complications described after the ASO for TGA. In a previous study we have shown that the pulmonary trunk and pulmonary sinus wall in unoperated hearts with TGA show a decrease of α-smooth muscle actin expression with increasing age. Thus the structure of the vessel wall and sinus wall of the aorta and pulmonary trunk are different in hearts with TGA. As described above, after the ASO the pulmonary root remains on the left side and becomes the neo-aortic root subjected to the systemic pressure. This may provide an explanation for the dilatation of the neo-aortic root that is sometimes observed late after ASO.

Apart from an abnormal and weaker α-smooth muscle actin expression of the wall of the pulmonary trunk and the disconcordant ventriculo-arterial connection described above, we hypothesize that in TGA the observed dilatation of the pulmonary root after ASO might also be due to histo-morphological defects.

Therefore, we studied the morphology of the pulmonary and aortic root in the left and right ventricles in hearts with TGA and compared our observations to the situation in normal hearts. We specifically paid attention to the amount of collagen and the distribution as well as myocardial support of the arterial roots using several histological markers. A three-dimensional (3D) anatomical reconstruction was made to show the relationship of the arterial fibrous annulus with the surrounding atrial and ventricular myocardium.
Material and Methods

Material
Four unoperated human heart specimens aged one day (postpartum) were studied including two with normal anatomy and two with simple TGA. The hearts were obtained from the Leiden Collection (Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands). This study is in accordance with the institutional guidelines of the Leiden University Medical Center for the use of human tissue.

Methods
All hearts were fixed in ethanol and glycerine and routinely processed for light microscopy. Transverse sections (10 μm) were mounted serially onto glycerine-coated glass slides. The paraffin-embedded sections were deparaffinated and stained with haematoxylin-eosin, resorcin-fucsin, modified Van Gieson and Azan for routine histological assessment of the specimens according to the institutional guidelines.

3D reconstruction
One normal heart and one heart with TGA were selected for 3D reconstruction based on the Azan-stained sections, providing good discrimination between collagen fibers, myocardium and vessel wall structures. The distance between the serial sections was 160 μm. Digital photomicrographs were taken of the serial sections of the normal heart and the TGA specimen from just below until just above the arterial roots. The lower limit was at approximately three quarters of the base-apex distance. The upper limit was determined amply above the sinotubular junction of the vessel wall, which is the upper limit of the collagenous annulus. The serial photomicrographs were incorporated into the AMIRA V4.0 software package (Template Graphics Software, San Diego, USA). The different cardiovascular structures (i.e. collagen, vessel wall, semilunar and atrioventricular valves and myocardium) were color-labeled by means of manual drawing and processed for 3D visualisation. Processing conditions of the normal heart and TGA specimen were similar, thus providing good conditions for direct comparison between the reconstructions.
Results

Below we describe the differences in collagen distribution, myocardial anchorage and embedding of the arterial root between the normal hearts and the hearts with TGA.

Collagen distribution
As described previously\textsuperscript{11,12}, in the normal heart the relationship between collagen and vessel wall at the most distal part of the annulus (commissure) is different from that of the mid-sinus region and the base of the sinus. For this reason we have compared the collagen distribution of the arterial root of the normal hearts to hearts with TGA at three different regions of the sinus: (I) at the level of the commissure (Fig. 1), (II) at the mid-sinus level (Fig. 2) and (III) at the base of the sinus (Fig. 3). 3D-reconstructions of both specimens show more clearly the distinct difference in collagen distribution of the annulus between the normal heart and the heart with TGA (Fig. 1-3a,b).

I. Commissure level
At the distal level in the normal heart, collagen fibers arising from the aortic annulus continue into the valve commissures on the inside of the aortic root and then merge on the outside of the elastic aortic wall with the adventitial layer (Fig. 1c,d). In TGA, the collagenous network forming the thin pulmonary annulus also concentrates distally to form the commissures. However, this layer does not extend any further to the adventitial layer surrounding the pulmonary trunk but only extends to the level just above the pulmonary annulus (Fig. 1e,f).

II. Mid-sinus level
At the mid-sinus level in the normal hearts the aortic sinus wall is surrounded by a thick collagen layer on the outside and by elastic lamellae at the luminal side (Fig. 2c,d). Compared to the normal hearts, in the TGA hearts the pulmonary trunk compact collagen layer on the outside is thin and both layers, elastic lamellae as well as collagen layer, are disorganized (Fig. 2e,f). In addition, the collagen layer in the TGA hearts has less extensive anchorage to the adventitial layer compared to the normal hearts (Fig. 2 compare c with e).

III. Base of the sinus level
At the base of the aortic and pulmonary sinus the elastic lamellae are absent in the wall of the sinus and the entire wall is composed of a collagen layer. In the normal hearts this
collagen layer is thick and extends into the surrounding myocardium via several finger-like protrusions forming a strong anchorage (Fig. 3c,d). In the TGA hearts similar to the mid-sinus region the collagen layer is thinner (Fig. 3e,f) compared to the normal hearts. The collagen layer extends finger-like protrusions into the surrounding myocardium, however, these protrusions are thinner and less extensive compared to the normal hearts (Fig. 3e,f).

Figure 1. Collagen distribution at the commissure level. 3D-reconstruction of the arterial roots of a normal heart in a right anterior view (a) and a TGA heart in a left anterior view (b). The amount of collagen (blue) is clearly decreased in the TGA heart. Transverse sections (c-f) of the arterial root stained with Azan showing the amount and distribution of collagen in the normal heart (c,d) and TGA (e,f). The intersection lines c in a and e in b refer to the respective sections below. At commissure level, in contrast to the aorta (Ao) in the normal hearts (c,d), in the TGA the collagenous network in the pulmonary trunk (PT) does not extend further into the adventitial layer (e,f). This collagenous layer in the TGA is interrupted by the elastic lamellae (compare arrow in d with f). Color codes: Atrial myocardium: light gray, Ao: transparent pink, Mitral valve (MV) transparent yellow, PT: transparent green. A: anterior, AoL: Ao lumen, L: left, LA: left atrium, P: posterior, PTL: PT lumen, R: right, RA: right atrium, RCS: right coronary sinus, RFS: right facing sinus. Scale bars: c,e = 600μm; d,f = 120μm.
Figure 2. Collagen distribution at the mid-sinus level. 3D-reconstruction (a, b) and transverse sections (c-f) as described in Figure 1. At the mid-sinus level the pulmonary trunk (PT) in the TGA shows a thin compact collagen layer at the outside, a less extensive anchorage to the adventitial layer and both, elastic lamella as well as collagen layers, are relatively disorganized compared to the normal heart (compare c, d with e, f). Color codes and abbreviations are similar to Figure 1. Scale bars: c, e = 200 μm; d, f = 120 μm.

Figure 3. Collagen distribution at the base of the sinus level. 3D-reconstruction (a, b) and transverse sections (c-f) as described in Figure 1. At the base of the sinus the collagen layer of the pulmonary trunk (PT) is thinner (e, f) and compared to the Ao in the normal heart (c, d) the finger-like protrusions (arrowheads in d and f) which attach the sinus with the surrounding myocardium, are thinner and less extensive (e, f). Color codes and abbreviations are similar to Figure 1. Scale bars: c, e = 200 μm; d, f = 120 μm.
Myocardial anchorage and support

Above we have described that in the normal hearts at the base of the sinus the annulus has a stronger anchorage to the surrounding myocardium via finger-like collagenous protrusions compared to the hearts with TGA. In this paragraph we describe the support of the roots by surrounding structures.

In the normal hearts the aorta is supported by its ‘embedding’ in the myocardium of the left ventricular outflow tract, ventricular septum and atrial myocardium forming a partial collar around it, while the pulmonary trunk is positioned more superficially on top of the right ventricle being only partially embedded by the myocardium of the right ventricular outflow tract and not being supported by atrial myocardium (Fig. 4a,c).

In the hearts with TGA most of the circumference of the pulmonary annulus seems to lie ‘on top’ of the left ventricular outflow tract, rather than being embedded into it (compare Fig. 4a and b). Only a small part of the pulmonary annulus circumference is supported by the left ventricular myocardium (Fig. 4d). Similar to the pulmonary annulus, the majority of the aortic annulus appears to be placed ‘on top’ of the right ventricular outflow tract and is thus not embedded in the surrounding myocardium (Fig. 4b,d). The atrial myocardium surrounds only a small part of the circumference of the pulmonary annulus compared to the aortic annulus of the normal hearts (compare Fig. 4a,c with b,d). Similar to the pulmonary annulus of the normal hearts, the aortic annulus has no support from the atrial myocardium (Fig. 4b,d).
Figure 4. Myocardial support. 3D-reconstruction of the arterial root and parts of atrial and ventricular myocardium of a normal heart in a right anterior view (a,c) and a TGA heart in a left anterior view (b,d). Transverse sections c and d are cranial views and correspond to the intersection lines c and d in respectively a and b. The aortic (Ao) annulus (blue) in the normal heart is supported by the atrial and ventricular myocardium forming a collar over most of its circumference (c). Both the Ao and pulmonary trunk (PT) in TGA appear to be placed on top of the heart instead of being surrounded by myocardium, as is seen in the normal heart (compare a with b). Only a small part of the circumference of the PT annulus is surrounded by myocardium (d). Color codes: Atrial myocardium: light gray, Ao: transparent pink, Mitral valve (MV) transparent yellow, PT: transparent green, ventricular myocardium: transparent dark gray. A: anterior, L: left, LA: left atrium, LV: left ventricle, P: posterior, R: right, RA: right atrium, RV: right ventricle.
Discussion

The main focus of the current study concerns the question as to what could be responsible for the sometimes observed dilatation of the neo-aortic root after ASO. Murakami and colleagues\textsuperscript{13} describe a decrease of distensibility at the base of the neo-aorta in post-ASO hearts, which they correlated with the disruption of vasa vasorum leading to the neo-aortic dilatation later.

In a previous study from our group we have shown that the arterial roots in TGA are structurally different from the arterial roots in a normal heart.\textsuperscript{10} In the unoperated TGA specimens, dedifferentiation of smooth muscle cells was observed, especially in the pulmonary root. This may be a factor leading to dilatation of the neo-aortic root following the ASO.

Although the pathogenesis of neo-aortic root dilatation after ASO may be in part explained by Murakami and colleagues\textsuperscript{13}, we postulate that the observed differences in collagen distribution and myocardial support of the arterial roots in TGA, as described in this study, as well as the structural differences of the roots in terms of the smooth muscle cells dedifferentiation\textsuperscript{10}, play a more significant role than the impaired blood flow of the vessel wall by disruption of vasa vasorum.

Collagen distribution

The results observed in this study confirm the findings as described by Bartelings et al.\textsuperscript{12} concerning the finger-like collagen structures, extending from the annulus, as well as their anchorage into the ventricular myocardium in the normal heart.

In the hearts with TGA, morphological differences were noticed compared to the findings in the normal hearts. An striking difference between the TGA and the normal heart specimens was the distribution and quantity of collagen in the arterial roots. The amount of collagen seems to be less in the hearts with TGA than in the normal hearts. Furthermore, in the TGA specimens, the collagen did not spread to the adventitial layer at the site of the commissures, whereas this was seen in both arterial roots of the normal hearts. Additionally, both arterial roots in the TGA specimens appear to be less firmly supported by the myocardium of the corresponding ventricles than both arterial roots in the normal specimens.

The development of TGA has been linked to neural crest migration abnormalities.\textsuperscript{14} Neural crest cells are involved in initiating the septation of the cardiac outflow tract.\textsuperscript{15} Exposing these cells to all-trans retinoic acid in animal models during development instigates several
cardiac malformations, one of them being TGA (16-19). In TGA mouse embryos, an altered collagen distribution was observed by Yasui et al.20. They conclude that treatment with retinoic acid and the subsequent neural crest abnormalities leading to TGA or TGA-like anomalies, induces the altered organization of extracellular matrix components, such as collagen type I and hyaluronic acid.

A more recent study by Jenkins et al.21 revealed malformed epicardium in retinoic acid receptor knock-out mice. The formation of the fibrous skeleton of the heart, which consists of collagen, is linked to epicardial differentiation and migration.22 When the epicardium is inhibited during development, the fibrous skeleton of the outflow tract is underdeveloped and its collagen appears to be distributed differently compared to the normal situation.23 Thus, the difference in collagen distribution between TGA and normal hearts, as observed in this study, may also originate from an epicardial problem during early development, since altered fibrous skeleton formation is also seen in retinoic acid receptor knock-out mice.

**Myocardial support**

In the normal heart, the aortic root is supported by atrial myocardium, left ventricular outflow myocardium and the ventricular septum forming a myocardial cuff surrounding it. The pulmonary root in the normal heart is not supported by the atrial myocardium and only the pulmonary infundibular myocardium bulges slightly over the pulmonary root, forming a modest collar surrounding it and providing some support and both roots are attached to the surrounding myocardium by finger-like protrusions.11,12

By studying the 3D reconstructions and the histological sections of the hearts with TGA, it is apparent that none of the roots is supported by the atrial myocardium. Both roots are placed more superficially ‘on top’ of the ventricles missing the sufficient myocardial cuff surrounding the roots as it was the case in normal heart. The attachment of the roots to the surrounding myocardium by finger-like protrusions was less extensive and thinner compared to the normal heart.

The retrospective study by Vandekerckhove et al.9, where 39 patients were examined 20 years after ASO, has revealed 60% dilatation of the neo-aortic root in the operated population. Long-term follow-up of this population is necessary to observe whether the remaining 40% develop dilatation of the neo-aortic root, which we relate to the morphological changes of the arterial root of the hearts with TGA.
Conclusions

In this study we observed an altered collagen distribution of the annulus at all three levels studied of both great arteries in TGA. Furthermore, both the aorta and the pulmonary trunk were found to be less firmly anchored to their corresponding ventricles in the TGA specimens than in the normal heart specimens.

We speculate that the findings in the present study, together with the observed structural differences between the arterial roots in TGA and normal hearts, in combination with subjection of the neo-aortic root to systemic pressures after ASO, might form the basis of the neo-aortic root dilatation that occurs after ASO.

Acknowledgements

We thank Jan Lens for expert technical assistance with the figures.
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References


