International Journal of Cardiology 177 (2014) 548–550

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Letter to the Editor

Inverse expression of estrogen receptor alpha and apolipoprotein B in coronary intimal hyperplasia of surgically repaired congenital heart disease: A pre-atherosclerotic condition?☆,☆☆

Rocío Castilla [a,1], Gabriel Cao [a,*,1], Rodolfo Kölliker Frers [a,1], Angélica Müller [a,1], Giuseppe Ambrosio [b,1], José Milei [a,1]

[a] Instituto de Investigaciones Cardiológicas "Prof. Dr. Alberto C. Taquíni", ININCA-UBA-CONICET, Buenos Aires, Argentina
[b] Division of Cardiology, University of Perugia School of Medicine, Perugia, Italy

ARTICLE INFO

Article history:
Received 25 June 2014
Accepted 17 August 2014
Available online 23 August 2014

Keywords:
Coronary intimal hyperplasia
Congenital heart disease
ERα
ApoB
Atherogenesis

Congenital heart diseases (CHD) or the process of their repair leads to an increased risk for adult cardiovascular disease compared with the general population [1]. The proliferation of intimal smooth muscle cells (SMCs), which causes intimal thickenings prior to any evidence of visible lipid deposition, was proposed to be the initial lesion of coronary atherosclerosis [2]. The increasing trend to consider intimal thickenings as possible pre-atherosclerotic lesions is based on the distribution of intimal hyperplasia in children and the localization of characteristic atherosclerotic lesions observed in adult humans [3]. Early coronary artery lesions may range from focal areas with mild myointimal thickenings in prenatal life to early soft plaques in infants, which may also present as intermingled lesions with components of both categories, more frequently observed with increasing age [4]. Also, we proposed that TGF-β1 expression might be considered another possible predisposing factor to develop atherosclerotic coronary artery disease in CHD patients [5]. On the other hand, estrogen receptor-α (ERα) appears to be responsible for the protective effects of estrogens against atherosclerotic vascular disease [6].

The aim of this paper was to analyze the immunohistochemical expression of ERα, TGF-β1 and apolipoprotein B in coronary intimal hyperplasia in children with CHD and the possible increased risk for the development pre-atherosclerotic lesions.

Coronary arteries were harvested from a total of 57 hearts of CHD patients who died at 6 days to 10 years of age (mean 2.5 years old, 50.8%, (n = 29) male). The main pathologies were tetralogy of Fallot, coarctation of the aorta and coronary artery anomalies, and for the most part they presented complex CHD. There were no statistical differences in the number of cyanotic (n = 30; 52.6%) vs. non-cyanotic cases (n = 27; 47.4%). Thirty-nine percent of patients (n = 23) underwent surgical repair of complex CHD and 78.3% (n = 18) of these surgical patients died within one month after surgery. Ten cases of pediatric patients aged ≤10 presenting arterial coronary intimal hyperplasia/thickening, but who died from causes other than CHD, were used as a control group. Samples of the four main coronary arteries were processed and thin sections for quantitative microscopy were stained with hematoxylin–eosin, Victoria blue (elastic fibers) and Masson's trichrome. Also, we performed immunohistochemical techniques against ERα (anti-ERα); TGF-β and apolipoprotein B (ApoB) using a modified avidin–biotin–peroxidase complex protocol and expressed as a percentage of positive area. Control sections were incubated with non-immune normal rabbit serum.

Image processing software (ImageJ, National Institute of Health, Bethesda, MD) was used for estimate: the maximal intima thickness, media thickness, and intima/media ratio. This ratio was used to assess the degree of intimal hyperplasia. Statistics was performed using Mann–Whitney test for non-parametric independent samples. Pearson coefficient was employed to determine correlation.

ERα expression was evident in all layers of arteries and was quantified as a percentage of the ERα-positive area, considering the whole artery, media or intimal layers. No significant differences between male and female CHD patients were found in the areas studied, neither between cyanotic and non-cyanotic, nor between controls and non-surgically-treated patients.

☆ The study received financial support from PIP 6549, CONICET and UBACYT M052, and University of Buenos Aires, Argentina, and from Institute S. Paolo, Turin, Italy.
☆☆ This work has been performed under a Framework Agreement between the University of Buenos Aires – Instituto de Investigaciones Cardiológicas – and the University of Perugia — Division of Cardiology.
* Corresponding author at: Instituto de Investigaciones Cardiológicas "Prof. Dr. Alberto C. Taquini" (ININCA), University of Buenos Aires, CONICET, Marcelo T de Alvear 2270, C1122AAJ Buenos Aires, Argentina.
E-mail address: gabrielcao@fibertel.com.ar (G. Cao).
[1] This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

http://dx.doi.org/10.1016/j.ijcard.2014.08.100
0167-5273/

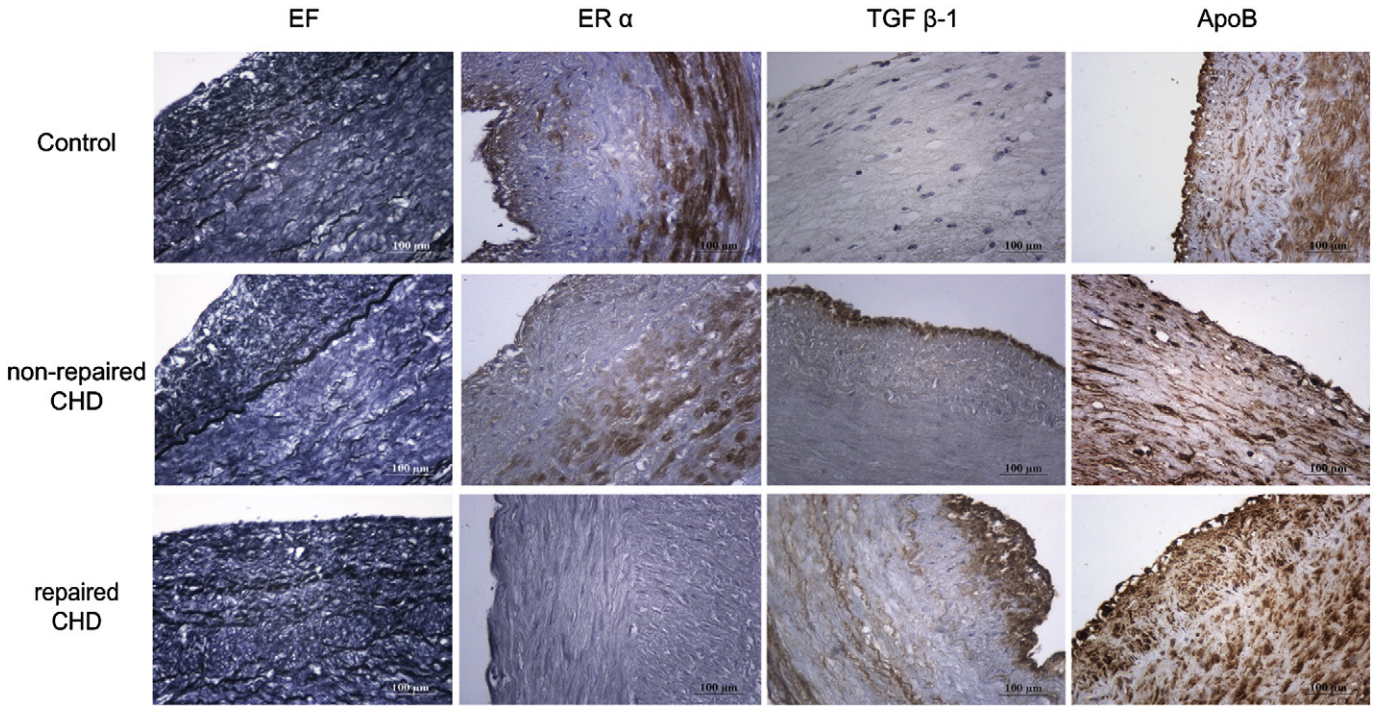

Fig. 1. Representative microphotographs of coronary wall stained with Victoria blue for elastic fibers (EF) from control, non-repaired and repaired CHD groups. Histologically, thickenings, and fragmented and duplicated internal elastic layer were equally observed among groups analyzed. Of note, in non-surgically repaired CHD, an increased immunohistochemical expression for ERα with corresponding reduction of TGF-β1 was observed at intimal and smooth muscle cell layers. In surgically repaired CHD these findings were reversed. Also, immunostaining for ApoB was increased over the thickened intima in repaired CHD compared with control and non-repaired CHD groups. These findings suggest an increased predisposition for coronary disease after surgically repaired CHD. Magnification ×400. Scale bar: 100 µm. CHD: congenital heart diseases; ERα: estrogen receptor alpha; TGF-β1: transforming growth factor beta 1; ApoB: apolipoprotein B.

A higher staining of ERα was evident in intimal thickenings from non-surgically repaired CHD patients, as compared to lower expression observed in surgically treated ones. Also, when this expression was quantified considering the whole artery, a significant decrease ($P < 0.05$) was observed in surgically repaired CHD patients (Fig. 1).

To avoid differences in artery size according to age, a similar methodology was applied in arteries whose intima/media rate was between 1 and 0.5 ($n = 42$). Accordingly, no significant differences in ERα expression in the intimal layer between male and female patients or between cyanotic and non-cyanotic CHD patients were observed. However, a significant diminution in ERα reactive area in this layer was evident in surgically treated patients vs. non-surgically treated. In all cases, the ERα expression in the media layer was unchanged. No correlation between degree of intimal hyperplasia (intima/media index) and percentage of intimal area stained with ERα was found (Spearman $r = 0.2444$, 95% confidence interval: −0.1530 to 0.5738; $P = 0.2100$).

ApoB cross reactive for B-48 and B-100 was found to be more positive over the thickened intima and around the intima-media transition in repaired CHD than non-repaired CHD alone ($P < 0.0001$) (Fig. 1).

Regarding TGF-β1 expression, no significant differences were found between cyanotic and non-cyanotic CHD patients (percentage of reactive area was 37.2 ± 12.2 and 25.9 ± 4.9, respectively). Furthermore, TGF-β1 expression was almost undetected in any of the 10 children that had no structural heart disease. On the contrary, when immunostaining for TGF-β1 was analyzed in patients with or without surgical repair, a very significant difference between them was observed (50.43% vs 15.91%, respectively; $P < 0.0005$). The nonparametric comparison of repaired CHD, non-repaired CHD, and controls without CHD revealed that the difference was significant ($P < 0.0001$). No correlation between degrees of intimal hyperplasia (intima/media index) and percentage of intimal area stained with TGF-β1 was found (Spearman $r = 0.2955$; 95% confidence interval: −0.61 to 0.11, $P < 0.134$). Table 1 summarized the analyzed immunohistochemical parameters.

The relationship between CHD and intimal thickening may involve different mechanisms, depending on the type of defect and surgical intervention. Endothelial hypoxia may occur either during the surgical manipulation process, and/or as a result of greater hemodynamic compromise (for example, increases in the oscillatory shear stress), being able induce hypoxia-inducible factor (HIF-1α) which could reduce the transcription of the ERα gene [7]; this may also explain the higher TGF-β1 and ApoB expression in CHD surgical patients [8]. Also, TGF-β1 and HIF-1α have been demonstrated to produce increased proteasome-dependent degradation of ERα in cancer cell lines [9]. If such mechanisms also operate in SMCs, they might explain the reduction in ERα expression in the intimal layer of these CHD patients. In conclusion, our results suggest that the intimal thickening in surgically

Table 1
Percentage of coronary artery wall area with positive expression for ERα and ApoB in the cases included in this study.

	Whole artery	Media layer	Intima layer	
	ERα (%)	ERα (%)	ERα (%)	ApoB (%)
Control	42.7 ± 3.7*	41.9 ± 4.8	45.05 ± 6.1#	10.6 ± 5.3
Non-repaired CHD	38 ± 5.0*	34.5 ± 4.5	27.4 ± 5.95‡	11.8 ± 6.2Ψ
Repaired CHD	23.1 ± 4.1	38.8 ± 4.5	10.5 ± 2.2	20.2 ± 5.4
Non- Cyanotic	31.8 ± 5.7	31.8 ± 5.6	21.5 ± 6.1	12.2 ± 6.3
Cyanotic	36.5 ± 4.6	36.5 ± 4.2	21.4 ± 6.5	11.3 ± 4.5
Female CHD	30.1 ± 7.4	41.9 ± 8.0	22.1 ± 7.6	10.8 ± 5.3
Male CHD	24.6 ± 3.3	41.9 ± 4.5	18.0 ± 3.8	11.2 ± 4.9

Values are means ± SD. Differences were analyzed using the Kruskal–Wallis test and Dunn's multiple comparison test.
CHD: congenital heart diseases.
ERα: estrogen receptor α.
ApoB: apolipoprotein B.
* $p < 0.05$ compare with repaired CHD.
# $p < 0.01$ compared with repaired CHD.
‡ $p < 0.05$ compared with repaired CHD.
Ψ $p < 0.0001$ compared with repaired CHD.

repaired CHD patient could be a consequence of a decrease in ERα expression in the intimal layer. For this reason, a reduction and increase in ERα and ApoB expression respectively might represent a risk factor for the development of pre-atherosclerotic lesions in coronary arteries.

Conflict of interest

There are no conflicts of interest.

References

[1] Kavey RE, Allada V, Daniels SR, et al. Cardiovascular risk reduction in high-risk pediatric patients: a scientific statement from the American Heart Association Expert Panel on Population and Prevention Science; the Councils on Cardiovascular Disease in the Young, Epidemiology and Prevention, Nutrition, Physical Activity and Metabolism, High Blood Pressure Research, Cardiovascular Nursing, and the Kidney in Heart Disease; and the Interdisciplinary Working Group on Quality of Care and Outcomes Research: endorsed by the American Academy of Pediatrics. Circulation 2006; 114(24):2710–38.
[2] Davies H. Atherogenesis and the coronary arteries of childhood. Int J Cardiol 1990; 28(3):283–91.
[3] Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2000;20:1262–75.
[4] Milei J, Ottaviani G, Lavezzi AM, Grana DR, Stella I, Matturri L. Perinatal and infant early atherosclerotic coronary lesions. Can J Cardiol 2008;24(2):137–41.
[5] Guerri-Guttenberg RA, Castilla R, Francos GC, Muller A, Ambrosio G, Milei J. Transforming growth factor beta1 and coronary intimal hyperplasia in pediatric patients with congenital heart disease. Can J Cardiol 2013;29:849–57.
[6] Rubanyi GM, Kauser K, Johns A. Role of estrogen receptors in the vascular system. Vascul Pharmacol 2002;38:81–8.
[7] Ryu K, Park C, Lee Y. Hypoxia-inducible factor 1 alpha represses the transcription of the estrogen receptor alpha gene in human breast cancer cells. Biochem Biophys Res Commun 2011;407(4):831–6.
[8] Leopold JA, Loscalzo J. Oxidative risk for atherothrombotic cardiovascular disease. Free Radic Biol Med 2009;47(12):1673–706.
[9] Schwartz SM, deBlois D, O'Brien ER. The intima. Soil for atherosclerosis and restenosis. Circ Res 1995;77(3):445–65.