The Coronary Microcirculation in Cyanotic Congenital Heart Disease

Eduard I. Dedkov, MD, PhD; Joseph K. Perloff, MD; Robert J. Tomanek, PhD; Michael C. Fishbein, MD; David D. Gutterman, MD

Background—Despite an appreciable increase in basal coronary blood flow in cyanotic congenital heart disease, flow reserve remains normal. We hypothesized that preservation of flow reserve resides in remodeling of the coronary microcirculation. Microcirculatory morphometric analyses were performed to test this hypothesis.

Methods and Results—Necropsy specimens from 4 sources were studied: (1) hearts from patients with Eisenmenger’s syndrome (A; n=5), (2) structurally abnormal hearts with ventricular hypertrophy (B; n=8), (3) structurally normal hearts with ventricular hypertrophy (C; n=6), and (4) normal hearts (D; n=5). To compare responses of the microcirculation to hypoxia versus hypertrophy, sections were taken from the left ventricular free wall, which in group A, was hypoxemic but not hypertrophied; in groups B and C, was hypertrophied but not hypoxemic; and in group D, was neither hypertrophied nor hypoxemic. Coronary arterioles were immunolabeled for smooth muscle α-actin. Measured morphometric parameters included long and short axes, area, and perimeter. Arteriolar length, volume and surface densities were calculated. There was a significant intergroup difference for arteriolar length density (P=0.03) and diameter (P=0.03). Total length density in group A hearts was markedly lower, but mean arteriolar diameter was significantly greater (34%) compared with group B (P=0.03). Arteriolar volume density was similar to that in the other groups.

Conclusions—Remodeling of the coronary microcirculation is the key mechanism for preservation of flow reserve in cyanotic congenital heart disease. The increase in short axis (diameter) compensated for lower arteriolar length density and was the principal anatomic basis for maintenance of normal flow reserve. (Circulation. 2006;114:196-200.)

Key Words: angiogenesis ■ cyanosis ■ heart defects, congenital ■ heart diseases ■ microcirculation ■ remodeling

The extramural coronary arteries in cyanotic congenital heart disease (CCHD) are typically dilated, often appreciably, sometimes aneurysmally, because endothelial vasodilator substances are elaborated in response to increased sheer stress of the erythrocytotic perfusate acting in concert with mural attenuation caused by inherent medial structural abnormalities. The increased basal flow in these dilated extramural coronary arteries might encroach on flow reserve. It is unlikely that preservation of flow reserve could be due to further dilatation of already maximally dilated extramural coronary arteries or to further oxygen extraction, which is already maximal. We therefore hypothesized that regulation of flow reserve might reside in the coronary microcirculation because of remodeling characterized by an increase in the number of resistance vessels (arterioles).

Clinical Perspective p 200

Acrylic resin casts in hearts of hypoxemic erythrocytotic residents acclimatized to high altitude disclose a striking increase in the number of secondary arterial branches leaving the main coronary arteries and in the number of peripheral ramifications. Hypoxemic erythrocytotic adults with CCHD might experience analogous remodeling of the coronary microcirculatory bed. Because morphometric analyses of the coronary microcirculation have not been done in CCHD, we compared precapillary coronary arterioles of hearts from patients with Eisenmenger’s syndrome with precapillary arterioles from hypertrophied but structurally normal hearts, hypertrophied and structurally abnormal hearts, and hearts that were structurally normal and nonhypertrophied.

Methods

Study Samples
Archival records of the University of California at Los Angeles Department of Pathology from 1998 to 2004 were reviewed to identify the 4 categories cited earlier: group A, hearts from patients with Eisenmenger’s syndrome (5 hearts); group B, structurally abnormal hearts with ventricular hypertrophy (aortic stenosis, hypertrophic cardiomyopathy; 8 hearts); group C, structurally normal hearts with ventricular hypertrophy (systemic hypertension; 6
hearts); and group D, hearts with no structural abnormalities and normal weights (no ventricular hypertrophy; 5 hearts). Eisenmenger’s syndrome was represented by nonrestrictive ventricular septal defect with suprasystemic pulmonary vascular vascular resistance and right-to-left shunt. Age at death in the 5 Eisenmenger’s syndrome patients was 32 to 44 years (mean, 36 years). Because our objective was to compare the response of the coronary arteriolar bed to hypoxia versus hypertrophy, sections were taken from the left ventricular free wall toward but not at the apex. This site in group A hearts was perfused by hypoxic blood but was not hypertrophied, because in Eisenmenger’s syndrome, left ventricular free wall mass was normal because of normal loading conditions, ie, normal aortic valve and normal systemic blood pressure; in groups B and C, the sections were hypertrophied but not hypoxic; and in group D, the sections were neither hypertrophied nor hypoxic (normal). Corresponding archival paraffin blocks were retrieved and utilized for immunolabeling and quantitative morphometry.

Immunohistochemistry and Immunofluorescence Microscopy

Nine-micron-thick tissue sections were deparaffinized, rehydrated, and immunolabeled with a monoclonal Cy3-conjugated anti–smooth muscle α-actin antibody, clone 1A4 (1:600; Sigma, St Louis, Mo). The sections were mounted with use of the Pro-Long Antifade Kit (Molecular Probes, Inc, Eugene, Ore). Fluorescence images were captured into a computer with use of a Nikon Eclipse E-600 microscope equipped with a Nikon DXM 1200 digital camera (Nikon, Melville, NY).

Quantitative Morphometry and Stereological Analyses

To determine the diameter, length, volume, and surface density of coronary arterioles (vessels with an uninterrupted smooth muscle α-actin–positive outline and an external diameter between 6 and 50 μm), we used Image-Pro Plus software (Media Cybernetics, LP, Silver Spring, Md) as described previously.6 Regions of myocardium, ~8 to 11 mm² per heart, were initially digitized under low-power magnification (objective ×2), and their areas were measured. By systematic scanning of these regions under high-power magnification (objective ×40), every vessel profile (without respect to its sectioning plane) was captured, and its morphometric parameters, including long axes, short axes (diameter), area, and perimeter, were measured. On the basis of these measurements, the length density (Lv) was calculated as \((2ab/N \cdot N/A)\) (in mm/mm²), where \(a\) = long axis and \(b\) = short axis of individual arterioles; \(N\) = total number of arteriolar profiles; and \(A\) = total area in which arterioles were measured.6 Volume density fraction (Vv) was calculated as \(A_{\text{total}}/A\) (in \(\mu m^3/\mu m^2\)) • 100%, where \(A_{\text{total}}\) = total area of arteriolar profiles, and \(A\) = total area in which arterioles were measured. Surface density (Sv) was calculated as \(P_{\text{total}}/A\) (in \(\mu m^2/\mu m^3\)), where \(P_{\text{total}}\) = total perimeter of arteriolar profiles and \(A\) = total area in which arterioles were measured. The morphometric analyses were performed by coauthor E.I.D., who was blinded to the sources from which the tissues originated.

Statistical Analyses

A 1-way ANOVA model was used to compare the 4 groups with respect to diameter, length density, volume density, and surface density. The groups were also compared according to the same model but with the results subcategorized by the size of the arterioles (6 to 15 μm, 16 to 25 μm, and 26 to 50 μm in diameter). When a significant intergroup difference was found, the results were subanalyzed with the Tukey-Kramer multiple-comparisons procedure and a global 95% confidence level to identify the cause of the difference. For length density variables, the data were logarithmically transformed to remove a significant right-tail skew and to satisfy one of the key assumptions of the ANOVA model. When the data were considered by diameter size, the results for the smallest vessels (6 to 15 μm) had to be logarhythmically transformed for all 3 outcomes. This was also the case for length density with the largest vessels (26 to 50 μm). The 2-sample \(t\) test was used for a basic comparison of Eisenmenger’s syndrome group with the average of the other 3 groups. Because of the small sample sizes in this study, the described analyses were checked with their nonparametric counterparts: the Kruskal-Wallis nonparametric 1-way ANOVA and the Wilcoxon rank sum test (as a substitute for the 2-sample \(t\) test and the Tukey-Kramer procedure, with an adjustment for multiple comparisons). All analyses were performed with the Number Cruncher Statistical System 2004 (NCSS, Kaysville, Utah).

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree with the manuscript as written.

Results

There were no statistical differences between the groups with regard to age and sex (Table). Heights and weights were not available because the ventricular specimens were secured from necropsy archives that did not store that information. The initial ANOVA model disclosed a significant intergroup difference for arteriolar length density and vessel diameter (Figure 1). No significant differences were found among the 4 study groups with regard to arteriolar volume and surface densities. These results were confirmed with the Kruskal-Wallis test. A subanalysis by the Tukey-Kramer multiple comparisons procedure disclosed that arteriolar length density in group A (Eisenmenger’s syndrome) was markedly lower than in group B (structurally abnormal hearts with ventricular hypertrophy). By contrast, the mean arteriolar diameter was significantly greater in group A than in group B. There were no other significant pairwise differences. The data indicated that arteriolar volume was similar in all 4 groups, although the power of the study to detect less than large-effect sizes was limited. Specifically, effect sizes of 0.74 or greater would have been detected with 80% power, and effect sizes of 0.85 or greater would have been detected with 90% power. It is possible, however, that the greater mean arteriolar diameter in the Eisenmenger’s syndrome hearts could have compensated for the lower arteriolar length density. In addition, the lower arteriolar length density in Eisenmenger’s syndrome hearts (group A) compared with structurally abnormal hearts with ventricular hypertrophy (group B) was mainly due to a markedly lower value for terminal arterioles (6 to 15 μm in diameter; Figure 2). It is evident that the higher mean arteriolar diameter for group A was exclusively due to the overall increase in arterioles >25 μm in diameter. The smallest (terminal) arterioles in hearts from Eisenmenger’s syndrome patients were sparse compared with the other 3 groups, but this deficit was compensated for by remodeling of larger arterioles 26 to 50 μm in diameter (Figure 3).

Discussion

Basal myocardial blood flow is appreciably increased in the right and left ventricular free walls and in the ventricular

Demographic Characteristics of the Study Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, y</th>
<th>Sex</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>32–46 (mean, 38)</td>
<td>3 male, 2 female</td>
</tr>
<tr>
<td>B</td>
<td>29–44 (mean, 33)</td>
<td>5 male, 3 female</td>
</tr>
<tr>
<td>C</td>
<td>36–48 (mean, 41)</td>
<td>3 male, 3 female</td>
</tr>
<tr>
<td>D</td>
<td>31–43 (mean, 39)</td>
<td>3 male, 2 female</td>
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septum in patients with CCHD, potentially encroaching on flow reserve. Because the extramural coronary arteries are in a state of chronic maximal dilatation with little or no capacity to dilate further and because their contribution to overall coronary vascular resistance is relatively small, we hypothesized that flow reserve is likely to reside in the coronary microcirculatory bed, which is capable of remodeling under a variety of vasculogenic/angiogenic stimuli.

Previous work on models of cardiac hypertrophy indicates that maximal coronary perfusion and coronary reserve are correlated with the net resistance in the microvascular bed. In volume-overload hypertrophy, for example, arteriolar length density and capillary numerical density were normal despite a marked increase in left ventricular weight. Minimal coronary vascular resistance during adenosine infusion was normal in these models. Enhancement of maximal coronary perfusion induced by exercise training is believed to result from coronary angiogenesis and/or arteriogenesis.

A key angiogenic stimulus for coronary vessel growth in hypertrophied hearts is the increased diffusion distance for O2, but myocardial specimens from our Eisenmenger’s syndrome (group A) hearts were taken from the left ventricular free wall toward the apex, a site that was perfused with hypoxic blood but was not hypertrophied. Hence, an increase in O2 diffusion distance could not have been an important angiogenic stimulus.

Growth and remodeling of existing microcirculatory blood vessels in Eisenmenger’s syndrome might occur in response to stretch as a mechanical trigger. Mural attenuation of the extramural coronary arterial walls in concert with elevated basal coronary blood flow could activate vascular endothelial growth factor (VEGF) and its receptors. Because the coronary arteries in CCHD are necessarily perfused by hypoxic blood, hypoxia per se might provoke elaboration...
of VEGF from myocardial smooth muscle cells, with upregulation of VEGF receptor-1 in heart endothelial cells.\(^9,10\) Supporting this mechanism are data from hypoxemic residents acclimatized to high altitude who have a striking increase in the number of secondary arterial branches leaving the main coronary arteries and in the number of peripheral ramifications\(^5\) and data from hypoxemic animals born at high altitude that have extensive myocardial capillary growth.\(^18,19\)

Figure 3. Coronary arterioles immunostained against smooth muscle \(\alpha\)-actin. In Eisenmenger’s syndrome hearts (A, C), the arterioles, especially terminal, are fewer in number compared with hypertrophied structurally abnormal hearts (B, D). However, the Eisenmenger’s syndrome heart arterioles are greater in diameter (E, G) than arterioles in hypertrophied structurally abnormal hearts (F, H). Scale bars are 50 \(\mu\)m.
A recent study of patients with CCHD disclosed reduced bioavailability of nitric oxide and impaired endothelium-dependent vasodilation in response to acetylcholine,20 observations that imply impaired nitric oxide–mediated angiogenesis21 that might account for the limited growth of terminal arterioles in Eisenmenger’s syndrome (group A). The lower arteriolar density in group A was associated with a 34% increase in arteriolar diameter, indicating that remodeling of arterioles upstream from terminal arterioles is a key mechanism that could account for the preservation of flow reserve in CCHD. Enhanced vasodilatory capacity of these resistance vessels may be a contributing factor.

Limitations
Arterioles were not prepared under controlled pressure because specimens were fixed by immersion rather than by vascular perfusion. The specimens of myocardium were taken from the left ventricular free wall toward but not at the apex, but more precise localization could not be achieved. Nor was it possible to match for variables such as sex, height, and body weight. Although the groups could not be matched for certain specific details of heart disease, they were matched with respect to ventricular hypertrophy and general category of heart disease. The study was powered to detect only relatively large-effect sizes, as noted previously.

Conclusions
Because basal coronary blood flow is appreciably if not maximally increased in CCHD and because myocardial O2 extraction is maximal or nearly so, we hypothesized that regulation of flow reserve resided in the coronary microcirculation, which compensates by remodeling in hypoxic erythrocytotic patients with CCHD as it does in hypoxic erythrocytotic residents acclimatized to high altitude. Microcirculatory remodeling emerged as a key contribution to the regulation of flow reserve. However, the lower length and volume densities, especially in terminal arterioles, implied that enhanced vasodilatory capacity supplemented remodeling.

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Disclosures
None.

References

CLINICAL PERSPECTIVE
The term “coronary artery” evokes the extramural coronary microcirculation. Less familiar to clinicians is the intramural coronary microcirculation, which is the subject of this report. In cyanotic congenital heart disease, the microcirculation remodels in response to hypoxemia. In the face of increased basal flow in the dilated extramural coronary arteries, vasculogenesis and angiogenesis serve to regulate coronary flow reserve, underscoring the interplay between the intramural microcirculation and the extramural microcirculation.
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