Coronary Capillaries in Patients With Congestive Cardiomyopathy or Angina Pectoris With Patent Main Coronary Arteries
Ultrastructural Morphometry of Endomyocardial Biopsy Samples

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Background. The coronary microvascularity may be abnormal even in the presence of angiographically normal epicardial arteries. Abnormalities of small coronary vessels have been invoked as a cause of angina.

Methods and Results. To quantitatively evaluate the morphology of capillaries in patients with idiopathic dilated cardiomyopathy (DCM) or the syndrome of angina and small vessel disease (SVD), we performed electron microscopic morphometry of capillaries in right ventricular biopsy samples taken from 32 patients. Ten had angina, normal epicardial coronary arteries, and evidence for SVD; 12 had DCM; and 10 had normal hearts. In patients with DCM, the ratio of microvessels to myocytes was not different than that of controls (0.49±0.06 versus 0.51±0.05). Mean cross-sectional areas of the capillaries (lumen plus wall) and lumen were significantly greater than those of controls (45.3±15.1 versus 22.7±8.3 µm², p<0.001; 17.6±6.9 versus 11.6±6.2 µm², p<0.05, respectively). Fibrous content of the myocardium, as assessed by quantitative light microscopy, was significantly increased (16.3±3.3% versus 5.0±2.4%, p<0.001). In contrast, in patients with SVD, the capillary-to-myocyte ratio was reduced (0.33±0.08, p<0.001). Although mean cross-sectional areas of the entire capillary (32.4±19.7 µm²) and the lumen (8.9±7.8 µm²) were not statistically different than those of controls, there was an absence of capillaries less than 15 µm² in cross-sectional area, and the frequency distribution of the lumen area was skewed to the left. Swollen endothelial cells frequently encroached upon the lumen. There was a mild increase in fibrous content (9.5±3.7%, p<0.05).

Conclusions. Enlarged capillaries and a normal ratio of capillaries to myocytes appear to be features of DCM. Of the patients with SVD, there was both a relative lack of capillaries and capillary lumen narrowing from swollen endothelium. These changes may induce ischemia and angina and may result in mild fibrosis. (Circulation 1991;84:203–210)

In a previous report,1 we described a group of patients with angina pectoris and angiographically normal epicardial coronary arteries. The endomyocardial biopsy specimens from these patients showed fibromuscular thickening of vessels less than 100 µm in diameter. Such findings have been previously reported in association with a variety of clinical conditions and have been termed “small vessel disease”2 (SVD). Histological abnormalities of small coronary vessels may be seen in association with hypertension, diabetes mellitus, amyloidosis, and autoimmune disease.2,6 In reports of patients with angina pectoris and patent epicardial coronary arteries, however, these clinical conditions were usually absent.1,7–9

Idiopathic dilated cardiomyopathy (DCM) is another condition in which the epicardial coronary arteries are typically normal, yet little is known about
vessels smaller than 100 μm in diameter, including capillaries in this condition.

Quantitative microscopy has been useful in characterizing the histopathology of the microvasculature of both cardiac and skeletal muscle in conditions such as diabetes mellitus, myocarditis, and various forms of cardiac hypertrophy.10–13 To our knowledge, no electron microscopic morphometric methods have been used to study capillaries in “idiopathic” SVD or DCM. We present our results of such an analysis. Biopsies from donor hearts taken before transplantation served as normal controls.

Methods

Patients

Thirty-two patients were studied; their ages and sexes are given in Table 1. All had a normal coronary angiogram, which was defined as no evidence of narrowing. Twelve patients had DCM. Ten patients had typical exertional angina pectoris, patent major coronary arteries, and histological evidence of idiopathic SVD, which was defined as narrowing of vessels less than 100 μm in diameter. Ten normal hearts served as controls; seven were cardiac allografts biopsied during transplantation, and three were from patients with episodes of atypical chest pain that proved to be noncardiovascular in origin. All patients gave informed consent for the endomyocardial biopsy.

Biopsy

The endomyocardial biopsy specimens were taken from the right ventricle with a Cordis Biop tome. Biopsy samples were placed immediately into 2.5% glutaraldehyde in 0.1 M cacodilate buffer. They were then cut into pieces and left in the same fixative for an additional 2 hours. After postfixation in buffered osmium tetroxide and dehydration in a graded series of alcohol, the pieces (two to six from each patient) were embedded in Araldite. Sections yielding the largest possible areas were cut from each block. They were stained with uranyl and lead and examined with a Philips 300 electron microscope.

Morphometry

In each block, a single section was chosen, and all myofibers and blood vessels in the section were counted and evaluated on the microscope screen. Capillaries were classified as normal or pathological with a subset of pathological findings classified as severely pathological (Figures 1–5). Pathological findings consisted of swelling and/or degeneration of the endothelial cells (Figure 3). Findings of endothelial cell necrosis with partial or total occlusion of the lumen by the swollen endothelium (Figures 4 and 5) were subclassified as severely pathological. The prevalence of pathological and severely pathological vessels and the ratio of vessels to myofibers were calculated for each patient.

Twenty to 60 capillaries were present in each biopsy specimen. Of these, 10–30 were photographed on 35-mm film and studied with computerized measurements. Vessels that were longitudinal and/or excessively compressed or twisted were excluded. The film negatives were projected at a known magnification onto a digitizing tablet (Summagraphic ID-1). Circumferential measurements were made with the aid of a cursor at the level of the inner and outer boundaries of the endothelial cells, the outer boundary of the basement membrane, and the outer perimeter of the entire capillary including pericytes. These data were fed into a PDP-11/34 computer.

One hundred eighty points on each perimeter were identified by the computer by calculating the coordinates of the intersection points of 180 equiangular radii emanating from the center of gravity of the lumen with lines connecting the original circumferentially placed traced points. The thickness of each layer was then measured along these radii between the newly found interpolated points. Mean values and SDs of the radii and the thickness of each layer were computed. For each vessel, five area calculations were then made: the cross section of the entire vessel as bound by the outer perimeter, the lumen, the endothelium, the basement membrane, and the pericytes. The ratios of the areas of the lumen, endothelium, basement membrane, and pericytes to the entire cross-sectional area were also calculated.

In all, 1,580 vessels were counted to determine the ratio of capillaries to myocytes: 615 vessels from DCM patients (51±17 vessels per patient), 545 from SVD patients (52±21 vessels per patient), and 420 from normal hearts (40±27 vessels per patient). One hundred fifty vessels from DCM patients, 112 from SVD patients, and 101 from normal hearts were examined by computerized morphometry.

To estimate the amount of fibrosis in the specimens, six to 12 sections from the paraffin blocks were stained with Van Gieson or Masson's trichrome stains. One-micron sections from two or three blocks of plastic-embedded material were also used. The slides were projected onto a television screen with a 100-point grid overlay. Point counts were made on four to 15 high-power fields.
Statistics

All values are expressed as mean±SD. Values for ejection fraction, prevalence of pathological vessels, ratio of vessels to myocytes, ratio of the areas of vessel components, and amount of fibrosis were compared among the three groups using one-way analysis of variance with Scheffe's F test. The absolute values for the entire capillary area and the luminal area were compared among groups using the Kolmogorov-Smirnov test because the distribution of these vessels in patients with SVD was not Gaussian.

Results

Men were more prevalent in all groups (Table 1). The controls were younger than the patients. In the SVD group, three patients had a past smoking history, and two of them also had mild-to-moderate hypertension. In the DCM group, one patient was a smoker, and another had mild hypertension. In the control group, one patient was a smoker.

Cardiac ejection fraction was 69.2±3.7% (range, 65–75%) for controls, 38.8±4.9% (range, 25–41%) for DCM patients (p<0.001), and 67.0±6.3% (range, 51–75%) for patients with SVD (p=NS).

Most of the microvessels in the biopsy samples were capillaries with walls comprising endothelial cells, basement membrane, and usually one or two pericytes. When observed, narrowing or occlusion of vessels was always a result of swelling of endothelial cells. No thrombi were seen. The morphological findings in vessels from smokers and/or hypertensive patients did not differ from those in vessels of other patients in the same groups.

In the control group, 6±4% of vessels were considered to be pathological, and 2±3% were severely pathological. The prevalence of pathological vessels was relatively low in the DCM group (16±11%, with 5±3% severely involved; p=NS) but significantly higher in patients with SVD (59±18%, with 37±12% severely pathological; p<0.001 versus controls) (Table 1 and Figure 6).

The ratio of microvessels to myocytes was 0.51±0.05 in controls, 0.49±0.06 in the DCM group (p=NS), and 0.33±0.08 in the SVD group (p<0.001 versus controls).

In the normal hearts, average capillary area was 22.7±8.3 μm², and mean lumen area was 11.6±6.2 μm². Individual values were normally distributed within the group (Figure 6) and within the individual patients. In the DCM group, cross-sectional areas were significantly increased; mean capillary area was 45.3±15.1 μm² (p<0.001), and mean luminal area was 17.6±6.9 μm² (p<0.05). The distribution of these values remained normal. In the SVD group, total capillary area was 32.4±19.7 μm², and luminal area was 8.9±7.8 μm². In this group, the values for the total microvessel and luminal areas were not normally distributed and differed significantly from those of controls (p<0.01) and DCM patients (p<0.001) when the Kolmogorov-Smirnov test was applied. As can be seen in Figure 6, some vessels in the SVD group had a severely reduced luminal area, with the distribution skewed to the left.
FIGURE 2. Electron micrograph of endomyocardial biopsy material taken from a patient with dilated cardiomyopathy; there is some artifactual compression, but the vessel is otherwise normal. L, lumen; E, endothelium; P, pericyte. Original magnification, \( \times 7,000 \).

Ratios of the areas of vessel components—lumen, endothelium, and basement membrane—to total vessel area are depicted in Figure 7. In DCM patients, ratios were similar to those of normal controls. In SVD patients, the lumen-to-total vessel area ratio was much smaller than that in normal controls (24.3±7.1\% versus 49.4±11.2\%, \( p<0.001 \)), whereas the endothelium-to-total vessel area ratio was much higher (65.1±11.2\% versus 30.1±6.0\%, \( p<0.001 \)), which is consistent with the marked endothelial swelling observed in many vessels. The ratio of the area occupied by basement membrane to total vessel area and the ratio of the pericyte area to total vessel area were similar in all groups.

An average of 11.2±2.6 fields per patient were evaluated to determine fibrotic content. The amount of fibrosis in the control group was 5.0±2.4\%. Biopsy samples from patients with SVD were mildly fibrotic (9.6±3.7\%, \( p<0.05 \)), and those from DCM patients showed marked fibrosis (16.3±3.3\%, \( p<0.001 \)).

Discussion

Histopathology of small coronary arteries has been identified in a number of disease entities, including hypertension,\(^4\) diabetes mellitus, amyloidosis, atherosclerosis,\(^2\) progressive muscular dystrophy,\(^3\) autoimmune disease,\(^6\) and cardiac allograft rejection.\(^5\) Small coronary vessel changes have also been reported in hypertrophic cardiomyopathy\(^4,6\) but not in DCM. Idiopathic SVD in patients with patent major coronary arteries has been previously described\(^7,8\) and is thought by some to be a cause of angina...
FIGURE 3. Photomicrograph of myocardial capillary showing mild pathology with swelling and degenerative changes in some endothelial cells (E). Lumen (L) is patent. Biopsy sample was taken from a patient with exertional angina and angiographically widely patent coronary arteries. Original magnification, ×7,000.

FIGURE 4. Photomicrograph of myocardial capillary showing severe endothelial cell pathology. Biopsy sample was taken from a patient with small vessel disease. E, endothelium; L, lumen. Original magnification, ×7,000.
pectoris.\textsuperscript{7-9} We reported on a group of patients with these findings\textsuperscript{1} and noted a slow flow in the epicardial coronary arteries, frequent supraventricular tachyarhythmias, and conduction disturbances that occasionally required implantation of a permanent pacemaker. The patients also had left ventricular wall thickening, an enlarged right ventricle, and reduced compliance of both ventricles. Histological assessment of endomyocardial biopsy samples showed abnormal microvessels with fibromuscular hyperplasia, myointimal proliferation, and endothelial abnormalities consisting of swollen degenerative cells encroaching on the lumen. Myofiber hypertrophy, lipofuscin deposition, and patchy fibrosis were also found.

In the present study, we quantitatively assessed the ultrastructural microvascular findings in endomyocardial biopsy samples from patients with DCM, patients with SVD, and patients with normal hearts. The observed ratio of microvessels to myocytes in right ventricular biopsy samples from normal hearts was similar to that reported byBillingham et al.\textsuperscript{19} In hearts from patients with DCM, this ratio was unchanged, although a possible increase in myofiber

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Electron micrograph of endomyocardial biopsy material taken from a patient with small vessel disease; shown are two myocardial capillaries with lumina (L) severely narrowed by endothelial swelling. Original magnification, $\times 10,000$.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Bar graphs of frequency distributions of capillary areas. Top row, total vessel area; lower row, lumen areas. Distribution is gaussian in control patients (N) and in patients with dilated cardiomyopathy (CM), whereas in patients with small coronary vessel disease (SVD), there is loss of gaussian pattern and a shift of luminal area distribution to left.}
\end{figure}
diameter would imply a relative lack of capillaries per volume of myofibers. In this context, it is noteworthy that the mean capillary area was increased, perhaps as a compensatory mechanism. The marked increase in the volume fraction of fibrosis found in DCM patients is consistent with previous reports. In patients with SVD, the vessel-to-myocyte ratio was low, suggesting microvascular destruction. The relative lack of capillaries as well as the high prevalence of abnormal capillaries may significantly impair oxygen delivery and result in angina pectoris. Furthermore, the mild increase in fibrosis in these hearts suggests that the impaired perfusion may have caused irreversible muscle injury, although there was no evidence by ventricular angiography or echocardiography for overt systolic dysfunction.

In the normal hearts, the distribution of values for total vessel area and lumen area was gaussian. Although the mean microvessel size in DCM patients was markedly increased, the distribution of values for vessel size remained normal. In contrast, the distribution of the values for total vessel area in SVD patients was skewed with values extending higher than in normal controls (more than 45 \( \mu \text{m}^2 \)) and an absence of vessels smaller than 15 \( \mu \text{m}^2 \) (Figure 6). Mean lumen area in SVD patients was not dramatically different from that of controls. However, examination of the histograms (Figure 6) shows that the distribution of the values for lumen area is skewed to the left and that many vessels have markedly narrowed lumens. Perhaps some spared vessels underwent compensatory dilatation, causing the wide range of values.

In the SVD group, the endothelium was the main target of pathological change. This confirms our previous qualitative impression that in idiopathic SVD, swollen endothelial cells cause a considerable narrowing of the lumen. Causes of endothelial swelling include ischemic, toxic, and immunological injury. Some of the ultrastructural changes in the SVD group resemble those seen in ischemia. Because the epicardial coronary arteries in our patients were widely patent, spams of the smaller arteries and arterioles might have been responsible for the microvessel ischemic changes. Mild spasm could become enhanced by release of vasoconstrictor substances (e.g., endothelin) from injured endothelium, and a cycle could thus be established, leading to further endothelial changes and angina. Small vessel pathology resulting from immunological reactions like those seen in autoimmune diseases or cardiac allograft rejection is also similar to the SVD changes described in the present study. However, our patients did not suffer from autoimmune disease and had normal blood globulins and erythrocyte sedimentation rates. Other conditions that could be responsible for endothelial damage, such as hypothalamic stimulation, alcohol, chemical toxins, and viral infections, do not appear to apply to the cases we present.

Similairties between hypertensive and idiopathic SVD have been described previously. Two of our SVD patients suffered from hypertension. Their ultrastructural characteristics were similar to those of the normotensive patients with SVD. Elimination of these patients from the SVD group did not change the statistical significance of the results.

There was no apparent cause, including selection bias, for the predominance of men in our series. In other studies of patients with angina pectoris and angiographically patent coronary arteries, both male and female predominance have been reported, as has been no sex predominance. The reason for this is unknown.

Limitations

Because of the nature of the biopsy procedure, only vessels in the subendocardium of the right ventricular septum could be examined. Thus, the described changes may not fully reflect the morphology of the remainder of the myocardium. Also, the vessels were not fixed in their distended, in vivo state and were therefore subject to artifactual changes of distortion, compression, and even tearing. Such changes were seen in all three groups. However, we excluded excessively distorted vessels from the present study. Furthermore, the specific capillary changes of endothelial swelling and cellular atrophy were not found in any of the biopsy samples from normal hearts, including those with vessels that were too distorted for morphometric analysis. Thus, the artifactual findings did not appear to overlap with the specific pathological changes of interest. Finally, for the present study, we focused attention on changes in capillaries and did not evaluate myofiber size; however, this information may be relevant to the interpretation of capillary fiber ratio.

Summary

We have described morphological features of coronary capillaries in patients with either DCM or SVD. In the former group, the capillaries were enlarged, with all components of vessel wall enlarged to similar degrees. In the patients with SVD, there
was evidence for capillary destruction. In addition, more than half of the capillaries had luminal narrowing resulting from endothelial swelling. These changes could lead to ischemia and be responsible for angina pectoris in patients with SVD.

References


KEY WORDS • coronary circulation • endothelium
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