Abnormalities of capillary microarchitecture in a rat model of coronary ischemic congestive heart failure


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Submitted 19 August 2014; accepted in final form 2 February 2015

Chen J, Yaniz-Galende E, Kagan HJ, Liang L, Hekmaty S, Giannarelli C, Hajjar R. Abnormalities of capillary microarchitecture in a rat model of coronary ischemic congestive heart failure. Am J Physiol Heart Circ Physiol 308: H830–H840, 2015. First published February 6, 2015; doi:10.1152/ajpheart.00583.2014.—The aim of the present study is to explore the role of capillary disorder in coronary ischemic congestive heart failure (CHF). CHF was induced in rats by aortic banding plus ischemia-reperfusion followed by aortic debanding. Coronary arteries were perfused with plastic polymer containing fluorescent dye. Multiple fluorescent images of casted heart sections and scanning electric microscope of coronary vessels were obtained to characterize changes in the heart. Cardiac function was assessed by echocardiography and in vivo hemodynamics. Stenosis was found in all levels of the coronary arteries in CHF. Coronary vasculature volume and capillary density in remote myocardium were significantly increased in CHF compared with control. This occurred largely in microvessels with a diameter of ≤3 μm. Capillaries in CHF had a tortuous structure, while normal capillaries were linear. Capillaries in CHF had inconsistent diameters, with assortments of narrowed and bulged segments. Their surfaces appeared rough, potentially indicating endothelial dysfunction in CHF. Segments of main capillaries between bifurcations were significantly shorter in length in CHF than in control. Transiently increasing preload by injecting 50 μl of 30% NaCl demonstrated that the CHF heart had lower functional reserve; this may be associated with congestion in coronary microcirculation. Ischemic coronary vascular disorder is not limited to the main coronary arteries, as it occurs in arterioles and capillaries. Capillary disorder in CHF included stenosis, deformed structure, proliferation, and roughened surfaces. This disorder in the coronary artery architecture may contribute to the reduction in myocyte contractility in the setting of heart failure.

coronary artery; capillary; congestive heart failure; hypertension; ischemia-reperfusion

IT IS WIDELY ACCEPTED THAT a decrease in intrinsic contractility of the myocardium is the primary defect in congestive heart failure (CHF) (5). CHF can be caused by many conditions that reduce the efficiency of the myocardium through damage or overload, including myocardial infarction, hypertension, valve defects, diabetes, arrhythmia, and genetic mutations (9, 28, 30). Coronary artery disease is the most common cause of CHF, comprising approximately two-thirds of patients with severe class IV heart failure (21, 25). However, the detailed mechanisms of reduced mechanical function remain unclear. Distinct defects of excitation-contraction coupling compartments and organelles have been identified in CHF cardiac samples and include changes in Ca^{2+} cycling proteins (4, 32, 37).

In this study we wanted to address the specific question of what happens to coronary architecture in rodent models in the setting of CHF. Transient (<30 min) arterial ligation, moderate occlusion, or blockage of a small vessel leads to submyocardial infarction (MI) and minor alternation or no change in cardiac function (11). Severe ischemia (>30 min) of a major coronary artery induces a large MI and serious heart dysfunction, usually due to pathological physical geometry: large MI reduce left ventricular (LV) ejection fraction (EF) and increase LV volume as myocardial mass is replaced by the noncontractile scar tissue (9). However, the remote myocardium is not ischemic. Therefore, how does contractile depression occur? In response to pressure overload or myocardial injury, the LV progressively remodels: remote noninjured myocardium hypertrophy and collagen fibrosis develop (20, 34). Hypertrophic myocytes usually have enhanced muscle contractility as long as blood supply is sufficient (26, 38). There is no enough evidence to show that deficient blood supply in remote myocardium post-MI or in pressure-overloaded hypertrophic myocardium leads to decompensation and CHF. In many cases, CHF has appeared in hearts with no history of MI or hearts that have endured a small MI. Use of inotropic agents in patients with CHF has been limited by adverse effects on outcomes because positive inotropic agents that increase intracellular concentration of cytoplasmic Ca^{2+} and enhance contractility are accompanied by increase of myocardial oxygen demands, leading to development, exacerbation, or intensification of ischemia and/or life-threatening dysrhythmias (2, 24). This phenomenon indicates that: 1) myocytes in CHF are reasonably responsive to beta-adrenergic drugs; and 2) the primary reason for energy starvation and decrease of myocyte contractility could be the reduction of blood supply reservation (14). Many studies in CHF patients have demonstrated impairment of coronary flow reserve and microvascular perfusion, via positron emission tomography (PET), single-photon emission computed tomography (SPECT), or contrast echo, and histological abnormalities of the microvasculature (23, 42). However, the systematic feature and role of abnormalities of coronary vasculature from the main artery to capillary have not yet been elucidated, especially in rodent model of CHF.

In the present study, we presume that arterial stenosis or neointimal narrowing is one of the compensatory mechanisms that attempt to reduce massive myocyte loss and improve general pumping performance by restricting injury to existing
ischemic areas while increasing blood supply to remote but relevant vital myocardium. Compensatory overshoot results in capillary disorder, which contributes to the reduction of vascular reservation and ultimately leads to impaired intrinsic contractility of the myocardium in ischemic CHF. The primary goal of current study is to explore a new approach to enhance understanding of the initial cause of CHF.

**METHODS**

All procedures were followed by the recommendations of the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services Publication No. NIH 78–23, 1996) and were approved by the Mount Sinai School of Medicine Animal Care and Use Committee.

**Animal protocol.** CHF was induced by consecutively performing aortic banding (Ab) for 2 mo, ischemia-reperfusion (I/R) for 1 mo, and aortic debanding (Ab + I/R + DeAb) for 1 mo as previously described (9). Briefly, Ab was performed in male Sprague-Dawley rats (150–180 g) by constricting the ascending aorta with a 4-0 suture against a PE-50 tube through right thoracotomy at the second intercostal space. Two months following Ab, rats underwent left coronary artery (LCA) ligation for 30 min followed by reperfusion to induce I/R injury. One month post-I/R, the rats underwent a third thoracotomy at the upper-right side of the sternum. The roots of approximately two to four ribs were cut along the right side of sternum to expose the

Table 1. Cardiac function by echocardiography in CHF rats

<table>
<thead>
<tr>
<th>Animal, n</th>
<th>AWd, cm</th>
<th>AWs, cm</th>
<th>PWd, cm</th>
<th>PWs, cm</th>
<th>EDV, ml</th>
<th>ESV, ml</th>
<th>EF, %</th>
<th>%FS</th>
<th>HR, beats/min</th>
<th>BW, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 10</td>
<td>0.21 ± 0.01</td>
<td>0.39 ± 0.01</td>
<td>0.21 ± 0.01</td>
<td>0.39 ± 0.01</td>
<td>0.55 ± 0.03</td>
<td>0.01 ± 0.00</td>
<td>97 ± 0.31</td>
<td>74 ± 2</td>
<td>379 ± 12</td>
<td>508 ± 27</td>
</tr>
<tr>
<td>CHF 17</td>
<td>0.21 ± 0.01</td>
<td>0.34 ± 0.02</td>
<td>0.23 ± 0.01</td>
<td>0.36 ± 0.02</td>
<td>0.97 ± 0.08**</td>
<td>0.18 ± 0.02**</td>
<td>80 ± 1.2.00**</td>
<td>45 ± 1.2**</td>
<td>351 ± 15</td>
<td>576 ± 13</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals. CHF, congestive heart failure; AWd, anterior wall, diastolic; AWs, anterior wall, systolic; PWd, posterior wall, diastolic; PWs, posterior wall, systolic; EDV, end diastolic volume; ESV, end systolic volume; EF, ejection fraction; FS, fraction of shortening; HR, heart rate; BW, body weight. **P < 0.01, compared with control.

aorta. The aortic banding suture was cut with microdissecting scissors and separated with microdissecting forceps. For all surgical procedures, anesthesia was induced by intraperitoneal administration of ketamine (65 mg/kg) + xylazine (13 mg/kg) + acepromazine (1 mg/kg). Animals underwent intratracheal intubation and mechanical ventilation.

**Echocardiography.** The animals were sedated by intraperitoneal injection of ketamine (40 mg/kg). Echocardiograms were performed as previously described (9). LV ejection fraction (LVEF) and fractional shortening data were obtained using M-mode.

**In vivo hemodynamics.** LV pressure was acquired and analyzed as previously described (9). Briefly, rats were anesthetized and maintained on isoflurane (1–2%). Thoracotomy was performed to expose the heart and provide access for an apical approach for pressure-volume conductance catheter placement (1.9 Fr; Scisense, Ontario, Canada). Preload response was induced by injecting 50 μl of saline into the jugular vein. This hypertonic saline bolus injection caused a transient increase of LV preload.

**Coronary artery resin casting.** After all other methods were completed, hearts were perfused with resin polymer using Batson’s No. 17 Plastic Replica and Corrosion Kit (cat. no. 07349; Polysciences, www.polysciences.com) as previously described (11). Proper fluorescent dye (www.blacklightworld.com) was mixed with red pigment (1:4 in weight) and added into casting polymer mixture. Briefly, while the rats were under ketamine anesthesia (90 mg/kg), the chest was opened, the right atrium was cut, and red latex (30 ml) was infused into the right ventricle. The frozen hearts were cryostated at 80°C. Samples for scanning electric microscope (SEM) imaging, histology, and myocytes were performed as previously described (10). For fluorescent imaging of vasculature, frozen sections were fixed with 3% glutaraldehyde and 1% paraformaldehyde in PBS, and photographed. Next, heart samples for section were cut into 5–6 pieces (each 2-mm thick), frozen in OCT and stored at −80°C. Samples for scanning electric microscope (SEM) imaging, heart was corroded in a 50-ml tube with 10 ml of Maceration Solution (no. 07359) at 50°C for 2–3 h and then room temperature for 24 to 48 h. After digestion, the cast was carefully washed in water and photographed.

**Scanning electric microscope.** Once dry, the coronary vascular casts were carefully mounted on an aluminum stub using double-stick carbon tape. Samples were then introduced into the chamber of the sputter coater and coated with gold in argon gas at 25 mA for 2 min. SEM examination was conducted with a field emission SEM (Hitachi; model no. S4300) at vacuum (3 keV, 10 μA, Flash 3).

**Histology.** The frozen hearts were cryostated at 8 μm. Masson’s trichrome, Picrosirisil red, and fluorescent staining of collagen fibers, and myocytes were performed as previously described (10). For fluorescent imaging of vasculature, frozen sections were fixed with 10% formalin for 5 min; the slides were mounted with DAPI medium after been washed three times in PBS. The capillary diameter was calculated with calibration of standard of Applied Image Analysis Micrometer.

**Statistics.** Variables are expressed as means ± SE. A Student’s t-test was performed to compare experimental groups using GraphPad Prism software. P < 0.05 was considered statistically significant.

## RESULTS

**Cardiac dysfunction in ischemic CHF.** Heart function was significantly decreased in CHF compared with control. The LV diastolic pressure in CHF hearts was dilated; EF and percentage of fraction of shortening decreased, but the LV diastolic anterior wall thickness in the CHF group was the same as the control group (Table 1).

**Coronary arterial pathological changes in the heart.** Overall, coronary vasculature volume increased from 0.055 ml/g of myocardium in control to 0.165 ml/g of myocardium in CHF hearts (Fig. 1, A–C). Arterial stenosis occurred mostly in postbranch segments (Fig. 1, D and E). Neointima formed not only in the main coronary artery [diameter (Ø): >350 μm] but also in middle arteries (Ø: >150–349 μm) and multiple small arteries (Ø: >50–149 μm; see Fig. 1, F–H). The MI size and degree of fibrosis in remote myocardium do not correlate with arterial stenosis. Neointima formed in the main coronary artery in 24% of CHF hearts, in middle arteries for 12% of hearts, and in small arteries for 29% of hearts; thus the overall incidence of arterial stenosis in all arterial levels is ~47% (Table 2).

### Table 2. Characteristics of ischemia-reperfusion injury, fibrosis, and arterial stenosis in CHF

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>MI Fibrosis</th>
<th>MA-S</th>
<th>Mid A-S</th>
<th>SA-S</th>
<th>Multi-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T***</td>
<td>+</td>
<td>@</td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>2</td>
<td>T**</td>
<td>+</td>
<td>@</td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>3</td>
<td>T*</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>4</td>
<td>T**</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>5</td>
<td>T**</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>6</td>
<td>Sub</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>7</td>
<td>Sub</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>8</td>
<td>T**</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>9</td>
<td>Sub</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>10</td>
<td>Sub</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>11</td>
<td>T**</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>12</td>
<td>T*</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
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<tr>
<td>13</td>
<td>Sub</td>
<td>+</td>
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<td>@</td>
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<td>14</td>
<td>Sub</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
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<tr>
<td>15</td>
<td>Sub</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>16</td>
<td>T***</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>17</td>
<td>Sub</td>
<td>+</td>
<td></td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>n = 17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T*** (12%)</td>
<td>8+</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>4T** (24%)</td>
<td>8+</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>3T* (17%)</td>
<td>1+</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@</td>
</tr>
<tr>
<td>8Sub (47%)</td>
<td></td>
<td></td>
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</tbody>
</table>

| Sum        | TMI 53      | 100% | 24%     | 12%   | 29%     | 29%     |

*n = number of animals. MI: myocardial infarction; T: transmural; Sub: no transmural; ***: MI >30%; **: MI 15–30%; *: MI <15% of left ventricle. Fibrosis: interstitial fibrotic area: + + +: >5%; + +: 3–5%; +: 1–2.9%; control <1% of nonischemic myocardium. MA-S: main arterial (Ø: >350 μm) stenosis; Mid A-S: middle arterial (Ø: >150–349 μm) stenosis; SA-S: small arterial (Ø: >50–149 μm) stenosis; @@@©©: 33–50%; ©©©©©©©©©: <15% of arterial internal area. Multi-S: multiple arterial stenosis; @@@©©©©©©©: vessel number >5; @@@©: vessel number 4–5; @@@©: vessel number 2–3.

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AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00583.2014 • www.ajpheart.org
Capillary density increased in remote myocardium. With the use of multiple fluorescent imaging for myocytes, collagen fibers, and capillaries in heart sections, capillaries with a diameter of 1–5 μm with cast resin were visible (Fig. 2). The structural relationship of myocytes and capillaries is not clear; some myocytes have six capillaries surrounding the muscle cell, while others have none (Fig. 2, C and D). There were 99 capillaries (Ø ≤ 10 μm)/mm² of myocardium in control, while there were 196 capillaries/mm² of myocardium in CHF. Capillaries in CHF hearts with diameters of 4–10 μm increased from 43 to 83%, while capillaries with diameters of 1, 2, and 3 μm were increased 10.6-, 3.9-, and 1.4-fold, respectively (Fig. 3, A and B) compared with control. This led to the increase of capillary cross area per milliliters squared of myocardium in CHF, especially for microcapillaries with a diameter of ≤3 μm (Fig. 3, C and D).

Characteristics of myocardial capillary morphology. Fluorescent imaging provided a basic snapshot of capillary cross sectional morphology (Fig. 2A), while a SEM was used to characterize capillaries in more detail longitudinally (see Figs. 4–7). Capillaries in the control group exhibited a primarily uniform arrangement, linear orientation, and straight, consistent shape; when they branch or change direction, they transition smoothly with minimal alterations in diameter. In contrast, capillaries in CHF hearts exhibited irregular arrangement, significant alterations in diameter, and curvy, distorted, inconsistent shape. Capillaries in CHF hearts had frequent narrow (Ø: <2 μm) vessel segments (Fig. 4). The length of capillary segments between two nodes where bifurcation occurred was shorter in CHF (46 ± 13 μm) compared with control (76 ± 8.6 μm; red lines in Fig. 4, A and C, and Table 3). Loci of capillary bifurcation in CHF exhibited greater multiplicity than control, with two or more branches splitting off a single node (Fig. 5). In addition, extremely narrow capillary branches (≤3 μm) appeared in CHF. These microchannels appeared to bridge between larger capillaries and contributed to the increase of microvascular density in CHF hearts compared with control (Fig. 3).

Fig. 2. Multiple imaging of myocytes, collagen fiber, and capillary in heart. A and B: fluorescent imaging of vessels and myocytes in CHF heart. Red, vessels; green, myocytes; blue, nucleus. C and D: double staining of capillary and collagen fiber for control (C) and CHF (D). Green, vessels; red, collagen fiber. Collagen fiber and capillary were significantly increased in CHF heart.
SEM imaging in CHF are summarized in Table 3. Capillary curling and deformation was found in all CHF samples. However, LVEF data from echocardiography are not linearly correlated to the degree of capillary disordersing (Table 3).

Coronary congestion and lower cardiac reserve. Chronic coronary congestion mostly occurred in capillaries, rather than higher level vessels, because coronary microcirculation cross-sectional area in the myocardium increased significantly with CHF compared with control (Fig. 8A). The LV was dilated in CHF. A transient elevation of preload induced by injecting 50 μl of 30% saline into the left jugular vein raised end diastolic volume and significantly enhanced contractility when myocytes were stretched, leading to increased EF in normal hearts. In contrast, in CHF hearts, the transient increase in preload resulted in a much smaller elevation in end diastolic volume and EF than control hearts (Fig. 8, B–D). These data indicate that lower cardiac reserve was probably associated with chronic congestion in coronary capillaries, which were likely not functioning well in CHF hearts based on SEM imaging.

DISCUSSION

Vasculature is a continuous system with flow from arteries, to arterioles, to capillaries, and finally back to veins. Pathological changes in the main coronary artery could be detected by angiography, PET, and magnetic resonance imaging (MRI) in vivo, while atherosclerosis in small arteries was detectable only by histological staining in vitro (12, 23). However, the role of capillaries in the development of CHF is not well known. In the present study, we report successive vascular changes of coronary arteries from main, middle, and small arterial branches to arterioles and capillaries in CHF, which were induced by chronic aortic constriction plus I/R followed with aortic debranching. We show that vascular pathological change was widespread, rather than isolated to a large artery such as the coronary left anterior descending artery, and successive, spreading from the main arterial stem through the capillaries.

The cardiac pumping function was affected by multiple factors: MI size, myocyte contractility, interstitial, and perivascular fibrosis, vascular pathophysiology, and neurohormonal factors (36). Coronary artery disease has long been considered one of the major causes of ischemic CHF (22). However, heart failure in the context of coronary artery disease is a heterogeneous condition. Many factors contribute to LV dysfunction, including stenotic severity, quantity of infarct-related arteries, subsequent remodeling hypertrophy, fibrosis, neurohormonal activation, systemic hypertension, myocardial revascularization, and endothelial dysfunction (21). Research thus far has focused on pathogenic changes in main coronary branches, such as plaque build-up and clotting in the left anterior descending artery (15, 40). Pathogenic changes occurring in small arterial or capillaries have not been studied closely enough due to shortage of proper animal models of coronary arterial atherosclerosis, especially in rodent hearts (1, 27, 29).

In our study, the data show that coronary structures in CHF exhibit not only vascular stenosis but also expansion. Both stenosis and expansion occurred not only in main coronary arterial branches but also in small arteries, arterioles, and capillaries. The mechanism of vascular stenosis/expansion in this model is not very clear. In chronic pressure overload (hypertension), a number of vasoconstrictors are released in response to increased sympathetic tone and activation of the renin-angiotensin-aldosterone system to maintain cardiovascular compensation in the midst of decreased cardiac output (5). It has been well documented that all those vasoconstrictors (catecholamine and angiotensin; Ref. 35) and cytokines (such as endothelins and tumor necrosis factor-α) contributed to neointimal formation (33), which could increase vessel resistance to blood flow (17, 41) to the overloaded myocardium.
The compensatory mechanism could be reversed and became deleterious when excess vasoconstrictors were released in a state of emergency, as these can rapidly increase oxygen demand or lead to insufficient blood supply (18). The compensatory overturn did not occur very often in the pressure overload model in the rodent if the aorta was barely constricted for a few months (9). When a transient occlusion of the coronary artery was added to the hypertrophic heart, an extra disturbance

![Fig. 4. Characteristics of capillaries in CHF. Images are taken with scanning electric microscope. A: control. Capillary thickness is uniform, and changes in diameter are smooth. B: CHF. Capillaries are curled, and changes in diameter are frequent and lack smooth transition. C: CHF. Capillary thickness varies drastically in CHF. The change in capillary diameter is irregular. Arrows indicate extremely narrow capillary branches. D: significant increase in number of smaller capillaries (Ø: <3 µm) in CHF compared with control. Total number of images = 209, of 7 control hearts and 7 CHF hearts. Segments between loci of capillary bifurcation are of significantly shorter length in CHF than in control (red line in A and C; see Table 3).](http://ajpheart.physiology.org/)

### Table 3. Characters of capillaries by scanning electric microscope imaging in CHF

<table>
<thead>
<tr>
<th>Animal Serial No.</th>
<th>AW TL, cm</th>
<th>EF, %</th>
<th>FS, %</th>
<th>Coronary Vessel/BW, mg/kg</th>
<th>Capillary Number Increase</th>
<th>Capillary Internode Length, µm</th>
<th>Capillary Curled, Distorted</th>
<th>Capillary Narrowed Irregularly</th>
<th>Capillary Bridge Increase</th>
<th>Capillary Coarse Surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHF-1</td>
<td>0.20</td>
<td>81</td>
<td>45</td>
<td>0.50</td>
<td>↑↑↑</td>
<td>40 (CNM = 196)</td>
<td>§§§</td>
<td>ØØØ</td>
<td>+ + +</td>
<td>@@E</td>
</tr>
<tr>
<td>CHF-2</td>
<td>0.19</td>
<td>75</td>
<td>41</td>
<td>0.45</td>
<td>↑↑↑</td>
<td>47 (CNM = 324)</td>
<td>§§§</td>
<td>ØØØ</td>
<td>+ + +</td>
<td>@@</td>
</tr>
<tr>
<td>CHF-3</td>
<td>0.17</td>
<td>80</td>
<td>44</td>
<td>0.19</td>
<td>↑↑</td>
<td>42 (CNM = 161)</td>
<td>§§§</td>
<td>ØØØ</td>
<td>+ + +</td>
<td>@</td>
</tr>
<tr>
<td>CHF-4</td>
<td>0.16</td>
<td>74</td>
<td>38</td>
<td>0.31</td>
<td>↑↑</td>
<td>68 (CNM = 89)</td>
<td>§§§</td>
<td>ØØØ</td>
<td>+ + +</td>
<td>@</td>
</tr>
<tr>
<td>CHF-5</td>
<td>0.17</td>
<td>79</td>
<td>43</td>
<td>0.15</td>
<td>↑↑</td>
<td>58 (CNM = 110)</td>
<td>§§§</td>
<td>ØØØ</td>
<td>+ + +</td>
<td>@</td>
</tr>
<tr>
<td>CHF-6</td>
<td>0.21</td>
<td>86</td>
<td>50</td>
<td>0.73</td>
<td>↑↑↑</td>
<td>32 (CNM = 577)</td>
<td>§§§</td>
<td>ØØØ</td>
<td>+ + +</td>
<td>@</td>
</tr>
<tr>
<td>CHF-7</td>
<td>0.23</td>
<td>68</td>
<td>34</td>
<td>1.43</td>
<td>↑↑↑</td>
<td>33 (CNM = 479)</td>
<td>§§§</td>
<td>ØØØ</td>
<td>+ + +</td>
<td>@</td>
</tr>
<tr>
<td>CHF (means ± SD)</td>
<td>0.19 ± 0.01</td>
<td>78 ± 2.2**</td>
<td>42 ± 1.9**</td>
<td>0.53 ± 0.17**</td>
<td>1- to 3-fold</td>
<td>46 ± 13**</td>
<td>See Fig. 4B</td>
<td>See Fig. 4C</td>
<td>See Figs 4 and 6</td>
<td>See Fig. 7, C and D</td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>0.21 ± 0.01</td>
<td>96 ± 0.62</td>
<td>75 ± 1.73</td>
<td>0.11 ± 0.02</td>
<td>—</td>
<td>76 ± 8.6</td>
<td>No</td>
<td>No</td>
<td>—</td>
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</tbody>
</table>

Total imaging numbers = 244; n = number of animals. CNM, capillary number of measured. §§§: Capillary curled, distorted in most observed area. ØØØ: Capillary diameter irregular in >50% of imaging area. ØØ: Capillary diameter irregular in around 30–50% of imaging area. + + +: Microchannel could be seen in most of imaging; + +: microchannel could be seen in around half of imaging; +: Microchannel could be seen in around a third of imaging. @@E: Coarse surface could be seen in most imaging at high magnification (≥1 k); @@: in around half imaging at high magnification; @: in around a third of imaging. **P < 0.01, compared with control.

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00583.2014 • www.ajpheart.org
in coronary vasculature was induced due to the simultaneous action of both systemic vasoconstrictors (neurohormonal factors) and local vasodilators (calcium overload, inflammatory factors, and oxygen radicals), which were infused after an I/R incident (19). Ischemic anaerobic glycolysis led to mass opening of capillaries, resulting in reduced resistance and slowed blood flow velocity (44). Vascular expansion and stenosis probably resulted from these paradoxical responses. It is not well known how arteries and capillaries were impacted during or after myocardial I/R injury in the context of chronic pressure overloading. The vascular proliferation induced by hypertrophy and ischemia contributed not only to neointimal formation but also to angiogenesis of capillaries.

Capillaries in the heart are affected by many factors, including cardiac afterload, ischemia-infarction, exercise training, high altitude, metabolism, and heart rate (7, 31). Rakusan et al. (39) reported that pressure overload LV hypertrophy in children demonstrated proportional capillary angiogenesis, whereas in adults hypertrophy seemed to be associated with failure of compensatory angiogenesis (6). Our data show that coronary vasculature volume increased from 0.055 ml/g of LV in control hearts to 0.165 ml/g of LV in CHF hearts, while capillary density in remote myocardium increased from 3.26% of myocardial cross-sectional area in control to 9.18% of myocardial cross-sectional area in CHF. Capillary growth could be a result of both pressure overload and I/R induced inflammation. Two features that indicate pathogenic capillary growth are 1) shortened capillary segments between nodes of vascular bifurcation; and 2) poly-bifurcations arising from one node (Figs. 4 and 5). These features could contribute to disturbed laminar flow, which is linked to artherosclerosis (8). With the use of SEM imaging, extremely narrow microchannels (Fig. 6) become visible in CHF hearts. These microchannels appear to form bridges between capillaries and seem to be distinct from narrowed capillaries (Fig. 4, C and D). The function of both microchannels and narrowed capillaries (diameter: ≈3 µm) were not clear but potentially could aggravate ischemia because it would be more difficult for red blood cells (diameter = 6.8 – 8.2 µm; Refs. 13, 16) to flow through the narrowed capillaries. The consequences of advanced vascular remodeling were detrimental in CHF hearts, not only due to the increase in capillary number, but also due to the alteration of functional structure. Increased capillary volume, caused by expansion and/or angiogenesis, led to cardiac congestion and LV wall higher tension, thus increasing wall stiffness and oxygen consumption, resulting in further ischemia and de-
crease in cardiac functional reserve (Fig. 8). Capillary disorder could be one of the mechanisms of inotropic agent side effects; this drug was initially used to enhance cardiac contractility to overcome heart depression but actually led to myocyte exhaustion (2), probably due to the capillary stenosis, which prevent myocytes from receiving adequate oxygen even though the main coronary arterial branches are not narrowed in chronic heart failure with coronary arterial disease (21). In contrast, mitigating capillary burden by reducing myocyte oxygen consumption, using agents such as β-blockers that inhibit LV contractility may relieve symptoms of CHF (30). Endothelin antagonists could also potentially improve the prognosis of CHF, since endothelial dysfunction in coronary capillaries is a crucial component of the progression of CHF (3).

The relationship between myocardial capillary density and coronary flow reserve in the CHF rats is not clear. This study does not compare the accuracy of the resin-casted fluorescent imaging with methenamine silver or lectin immunohistochemical staining for capillary density (39, 43). The data in current study for capillary blood pressure, flow velocity, or resistance in the coronary vasculature are also not available mainly due to technical barriers. While the data show that the innermost capillary surfaces are visibly roughened and textured, we could not conclude a cause-and-effect relationship between endothelial disorder and myocyte dysfunction. The present study also lacked data regarding the levels of humoral factors such as endothelin, angiotensin II, norepinephrine, and vascular endothelial growth factors, which were probably involved in the pathogenesis of capillary disorder. More work is required, both in vitro and in vivo, to better understand the role of capillary disorder in CHF. The present study’s findings need to be expanded upon utilizing updated cardiac imaging techniques and in-depth analysis of abnormalities on biochemical, molecular, and genetic levels.

Summary. In a chronic pressure overload plus I/R model of heart failure, we found that vascular disorder occurs not only in main branches of the coronary artery but also in arterioles and capillaries. The myocardial capillary density did not directly correlate to cardiac functional improvement because there is an intermediate factor which plays a critical role in material exchange between blood and myocytes: capillary structure. The capillary structural disorders found in CHF hearts include stenosis, nonlinear arrangement, curled shape, drastic changes in diameter, proliferation, and roughened surface texture. This disorder might be one of critical contributing factors to the energy starvation, which leads to reduction of intrinsic con-

Fig. 6. Microchannel formation between capillaries in CHF. A: there are very few microchannel (Ø: <3 μm) connections between capillaries in control. B-D: microchannels were opened in CHF. It was not clear that those microchannels were “opened” or “angiogenesised.”
tractility of myocytes. Further research needs to incorporate technological advances of molecular biology in conjunction with physiological measurements, such as PET, MRI, and myocardial contrast echocardiography, to best elucidate the role of the coronary microvascular dysfunction in ischemic CHF.

**GRANTS**

This work was supported in part by National Heart, Lung, and Blood Institute Grants R01-HL-093183 and P20-HL-100396 (to R. J. Hajjar) and K23-HL-111339-01 (to C. Giannarelli) and a grant from the Transatlantic Leducq Foundation (to R. J. Hajjar).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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**REFERENCES**


