The Microcirculation of the Human Heart: End-capillary Loops with Discrete Perfusion Fields

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SUMMARY We studied 10 autopsied human hearts by perfusing colored Microfil into separate coronary arteries to define organization of capillaries at the borders between two perfusion fields. Sections of “cleared” myocardium were examined with epilumination at the grossly identified borders of Microfil perfusion. In two- and three-color-injected hearts, the capillaries were arrayed in a pattern of arcades and loops without connections between separately perfused capillary beds. In hearts perfused through only one coronary artery, the capillaries were organized into tufted loops at the border. These findings contrast with the microcirculatory pattern in canine skeletal muscle and brain, in which heterologous capillaries are focally interconnected. We conclude that the human microcirculation is composed of end-capillary loops that supply discrete perfusion fields. This pattern of unconnected heterologous capillary beds suggests that there is no obvious anatomic arrangement of the microcirculation that could account for a significant ischemic lateral border zone in human myocardial infarctions.

THE LATERAL border zone of acute myocardial infarction has been an area of intense interest over the past decade. The possibility that appropriate interventions may salvage ischemic myocardium otherwise destined for necrosis in this region motivated this attention. The existence of such a zone, however, has been accepted by many investigators without regard to the anatomic arrangement of the coronary circulation that would allow lateral regions of the myocardium to survive in an ischemic state. Since proximity to capillaries supplying oxygenated substrate is a major determinant of cellular viability, delineation of the myocardial microvasculature is crucial for understanding this lateral zone.

The anatomy of an acute myocardial infarction generally has been depicted as a “bullseye,” with a central region of necrosis surrounded by a lateral and subepicardial border zone of ischemic but viable tissue that blends gradually into the normal myocardium.1, 2 For there to be a significant lateral border zone based on the anatomy of the coronary microvasculature, one would have to postulate an arrangement of the microcirculation organized in one of two ways (fig. 1): Either there would have to be a pronounced interdigitation or overlapping of capillaries derived from two separate larger vessels upstream, so that occlusion of one artery would allow cells to be supplied with diminished but sufficient substrate from the capillaries supplied by the nonocluded artery; or the capillaries derived from two major vessels would have to be intimately connected at the tissue level, so that again, cells would be partially supplied by the branches derived from the patent vessel.

In studies of normal dogs,3 we showed that the capillary circulation in the lateral border region is composed of end-vessel loops and arcades without cell to cell overlapping, and without anastomoses between capillaries arising from two separate large arteries. The anatomy of the microcirculation in the canine heart is discrete and the vessels supply a specific volume of myocardium. The absence of overlapping and interconnecting microvessels is consistent with the absence of a lateral border zone in 24-hour canine infarcts studied with serial section histology,4 or histology combined with vascular identification.2 Other investigators, using different techniques, also have suggested that the lateral border zone is insignificant in size.6-12

Despite considerable interest in the border zone concept in human myocardial infarctions, and extensive knowledge of interarterial anastomoses above the arteriolar level, little information is available about the anatomy of the human cardiac microcirculation. In this report, we describe the nature of the human capillary anatomy and, specifically, determine whether it is organized as in the dog or arranged with interconnections or interdigitations that might support an ischemic but surviving lateral border zone.

Material and Methods

Human Hearts

Ten adult (mean age 61.7 years) human hearts were obtained at autopsy 8–12 hours after death. The cases were randomly selected over a 6-week period, and are representative of patients coming to postmortem examination in our general hospital. Eight patients had primary diseases that did not specifically involve the heart (three cases of diabetes mellitus and sepsis, one case of diabetic ketoacidosis, one Hodgkin’s disease, one subarachnoid hemorrhage, one ruptured aortic aneurysm and one alcoholic cirrhosis with lung abscess). Of the two patients with cardiac disease contributing to death, one chronic alcoholic had infectious endocarditis of the tricuspid valve and one had an old and acute myocardial infarction with distal thrombotic occlusions of the right and left circumflex coronary arteries. In the latter case, all three major coronary arteries were perfused, but only the beds of the left anterior descending and the proximal right and left circumflex arteries filled.
Plastic cannulas were placed into the proximal coronary arteries and were anchored to the vessel with ligatures. In two cases, significant proximal coronary atherosclerosis precluded perfusion of more than one vessel, and therefore only the left anterior descending coronary artery was cannulated. In five cases, the left anterior descending and the left circumflex arteries were perfused, while in three cases these two vessels were perfused along with the right coronary artery. Separately colored silicone rubber solutions (Microfil, Canton Bio-Medical Products, Inc.) were used for each vessel. Before perfusion, heparinized saline was flushed into the coronary arteries until the heart became pale and the coronary sinus effluent was clear. The vessels were perfused with Microfil simultaneously and under the same pressure (100 mm Hg) to minimize cross circulation through large, preformed epicardial collaterals. We described our method for multiple, simultaneous vessel perfusion previously.5

After perfusion, the Microfil was “cured” by placing the hearts in ice for several hours. They were then fixed in 4% buffered formaldehyde. The ventricles were sliced into 1-cm horizontal rings from apex to base, and rings with adequate microvascular filling (judged by the diffuse and relatively homogeneous coloration of the tissue by the silicone rubber) were cleared by the method of Schaper.13 The procedure results in the transformation of the myocardium into semitranslucent, amber tissue with colored zones corresponding to the Microfil-perfused microvasculature. Portions of tissue 1–2 mm thick were removed freehand from regions where two colors abutted. In single perfused specimens, sections were taken from the border of the colored zone. Tissue slices were examined in a standard light microscope and were photographed with epillumination to visualize the color of the Microfil within the microcirculation. Border regions from all 10 specimens, and from at least two ventricular slices within the same specimen, were examined and photographed.

Canine Tissues

To validate our technique and to compare the microcirculatory bed in different tissues, we studied hearts, skeletal muscle and brain from dogs. The microcirculation of the dog heart has been described;3 the absence of coronary lesions in the dog make it useful for comparison with the human hearts used in this study, several of which had moderately abnormal coronary arteries.

Gracilis skeletal muscle from the hindlimb was chosen to allow easy access to the supplying vessels. Eight specimens were perfused with Microfil using the same technique described for the heart. The main femoral artery was perfused with one color in all eight muscles, and a muscular branch was perfused simultaneously with a different color in five specimens (three muscles were singly injected). The tissues were cleared and examined microscopically, as described for the heart.

Cerebral perfusions adequate for evaluation of the microcirculation were more difficult than those for either heart or skeletal muscle. Postmortem cerebral edema apparently limits the ability to fill small vessels consistently with silicone rubber. To obviate this problem, we perfused two brains with heparinized saline plus mannitol (5%) before using Microfil. The basilar and middle cerebral arteries were perfused simultaneously under controlled pressure with red and white Microfil, respectively, and the tissues were cleared. Sections from the superficial cortex, where filling was optimal, were selected from regions in which the red and white Microfil abutted.

Results

Human Hearts

In the eight specimens which were doubly or triply perfused with Microfil, sections examined from areas where two colors abutted (a region equivalent to the lateral border zone in infarcted hearts), were sharply demarcated (fig. 2). There was no evidence of connections across the border; microvessels filled with one color of silicone rubber remained discrete from equivalent vessels filled with another color. This separation between two vascular beds could only be appreciated with epillumination of the field. Transillumination of the tissue, in which Microfil appears black regardless of color, did not permit identification of discrete vascular beds.

Along the border, capillaries were frequently organized into loops. Vessels approached the abutting vascular field and looped back on themselves with sharp hairpin-like turns, often coming within several microns of loops filled with a different color. At higher magnifications, we could see that loops often had
straight extensions at their apices, which formed one or a series of other loops organized as an arcade (fig. 3). The distance between the two arms of the loop was sufficient to surround a single myocardial cell. This can be appreciated in a specially stained section of human myocardium in which the capillary walls are visible (fig. 4).

Not only were individual capillaries arranged as loops or organized into an arcade system with no obvious connection to heterologous loops and arcades, but in any one field along the border, the microcirculation was relatively homogeneous; that is, there was no pronounced interdigitation or overlapping of microvessels filled with different colors of Microfil, sufficient to provide individual or small groups of cells with substrate derived from two separate larger vessels. With rare exception, we also did not see areas of the microcirculation with two colors in the same vessels. In the few cases in which double filling was noted in small regions of the microvasculature, a large vessel containing two colors of silicone rubber was consistently identified in the same area. This suggests that the source of mixing was above the microvascular bed, probably at the level of preformed epicardial collaterals.

In the two specimens perfused through only one vessel, a loop- and-arcade anatomy was apparent at the periphery of the vascular field (fig. 5). The silicone rubber did not diffuse irregularly into the nonperfused zone, as one would expect if there were connections between the vascular beds; rather, the capillaries ter-

**Figure 2.** A low-power photomicrograph of the vascular border between two separately perfused capillary beds. The light-colored vessels were filled with yellow Microfil and the less distinct vessels in the darker zone were perfused with red Microfil. The border is irregular, but there is a sharp demarcation between the red and yellow zones. Even at this magnification, numerous capillary loops (arrows) can be appreciated. There are no apparent heterologous capillary connections across the border, and individual vessels are not double-filled with two colors of Microfil. Both capillary beds are intimately abutting, yet they remain entirely discrete. Epiilluminated, cleared tissue; magnification × 63.

**Figure 3.** A white Microfil-perfused arcade approaches within several microns of a red-filled capillary bed (RC), at which point the white vessels loop back on themselves. Loops often have straight extensions at their apices (arrows) that form additional loops in an arcade pattern or which end abruptly. The latter may result from sectioning artifact or incomplete Microfil perfusion (see Discussion). Note the absence of connections between the red and white capillaries. Epiilluminated, cleared tissue; magnification × 158.
hearts were a consequence of multiple interdigitating peninsulas. In the doubly perfused specimens of this investigation, we frequently found discrete vascular peninsulas supplied by vessels with one color of Microfil invaginating into tissues supplied by a different large vessel (fig. 6). Again, within the peninsulas, we observed capillary loops without evidence of interconnection to the surrounding microcirculation, despite the proximity of the discordant vessels.

This description of the human cardiac microcirculatory anatomy should not be interpreted as indicating that heterologous capillaries (supplied by two separate larger vessels) are uniformly arranged in an abutting end-to-end loop pattern. In fact, along the vascular border, we often found closely apposed parallel arrays of two separately perfused groups of capillaries oriented longitudinally. This was particularly obvious at the periphery of interdigitating peninsulas supplied by one set of capillaries at the interface with the surrounding capillary bed. In contrast to an anastomosing microvascular system where one might expect to observe interconnected side branches filled with two colors of Microfil (see skeletal muscle and brain, below), the capillaries remained discrete, and no obvious heterogeneous perfusion was seen within individual vessels. Thus, regardless of the capillary orientation with respect to a heterologous microvascular bed in the heart, there is no evidence of interconnection by either a loop-to-loop bridging anastomosis or a side-to-side arcade network.

Canine Skeletal Muscle and Brain

Perfusion of gracilis muscle with two different colors of Microfil revealed findings generally similar to those in the dog and human hearts. The border between two vascular fields was relatively discrete, with arrangement into a loop-and-arcade anatomy. However, in contrast to myocardium, clear interconnections were observed focally between the two capillary beds (fig. 7). In such instances, microvessels within a loop or arcade network were double-filled with both colors of Microfil; or occasionally, a single branch extended from the apex of one loop to that of another filled with a different color. Thus, the microcirculatory bed of the canine gracilis muscle is focally interconnected. We did not observe marked overlapping or interdigitation of the two separate vascular beds.

In the doubly perfused brain specimens, the superficial cortical microcirculation was diffusely interconnected, with virtually every arcade associated at some point with a discordant arcade. Within the vessels, the Microfil perfusate was mixed, with two colors apparent. Distinct loops were not usually observed.

Discussion

In this study, we have demonstrated that the human cardiac microcirculation is arranged in the form of discrete end-capillary loops and arcades, without interconnections to capillaries supplied by a different large vessel. We also showed that the vascular border is
Figure 6. A red Microfil-perfused peninsu-la (dark zone with relatively indistinct vessels) invaginates into a region of the lateral border supplied by white filled vessels. The white microvascular bed has been sectioned obliquely, which accounts for many of the short-vessel profiles in the field. Note the tufted appearance of the capillary loops (arrows) viewed on end. There is no evidence of connection between the red- and white-filled zone, nor are there double-filled vessels in this region. Epiilluminated, cleared tissue; magnification × 63.

Figure 7. A portion of canine gracilis muscle perfused with red and white Microfil, simultaneously and at the same pressure, into the femoral artery and a muscular branch. The capillaries have a wrinkled appearance because of muscle shortening during fixation. There are numerous separate white (W)- and red (R)-filled capillary arcades and loops that anastomose focally (arrows). Within the anastomosing branches there is either a mixture of white and red Microfil or the two colors abut within the same vessel. This evidence of focal microvascular anastomoses was also observed in the superficial cerebral cortex, but was not seen in the human hearts, although the perfusion methods were similar in all tissues studied. Epiilluminated, cleared tissue; magnification × 158.
irregular, with peninsulas supplied by vessels that remain separate from those supplying surrounding tissues. Despite this irregularity of the border region, however, individual or small groups of myocardial cells are not supplied by capillaries arranged in an alternating or overlapping pattern sufficient to provide substrate if a large feeding vessel was occluded. The findings are identical to those we have described in the normal dog heart, and they may be characteristic of all mammalian species.

In contrast to the discrete microcirculation of the dog and human hearts, the capillary beds of canine gracilis muscle and brain are interconnected. Double filling of microvessels in skeletal muscle was observed focally, as were bridging vessels extending between heterologous capillary loops. Despite this focal vascular crossover, however, the microcirculatory beds were relatively homogeneous. In the superficial cerebral cortex, though, diffuse double filling of capillary arcades was apparent, suggesting more extensive interconnections between the vessels. Since both of these studies in skeletal muscle and brain were performed identically to those in the heart, we believe that the different findings reflect differences in anatomy rather than artifacts of the perfusion method.

Although we had surprisingly good success with our postmortem perfusion technique, considering the unstandardized nature of the hearts selected for study, some methodologic problems might have affected the results. It was not unusual to find incompletely filled loops or straight extensions from the apex of a loop that ended abruptly. Incomplete filling may represent inadequate tissue perfusion due to vessel obstruction (i.e., air emboli or platelet thrombi), or it may result from sectioning artifacts. We also noted small areas of myocardium completely devoid of Microfil, but we tried to select regions for evaluation that had two well-filled groups of closely abutting vessels. Focally poor tissue perfusion could obscure heterologous capillary anastomoses; however, it is unlikely that such connections would be limited to these areas. The fact that they were not observed in the well-perfused zones suggests that our results are representative of the tissue as a whole. In addition, perfusion defects in canine skeletal muscle and brain did not preclude the demonstration of double-filled microvessels or obvious intercapillary anastomoses.

Although overt coronary disease was present in only two of the 10 hearts, some degree of coronary obstruction may have been present in several of the other hearts. In addition, the technical factors discussed above undoubtedly contributed to focal pressure inequalities during the perfusion of Microfil into the coronaries. The procedure, using equal and simultaneous pressure perfusion, was designed to limit cross circulation between large preformed collaterals. Any pressure inequalities downstream, because of anatomic lesions or technical artifacts, should have enhanced the possibility of observing connections between the terminal vascular beds, and yet the connections were not observed. The absence of mixed colors in the terminal bed found in a series of heterogeneous human hearts is surprising, unless the terminal vascular bed is poorly interconnected.

We infrequently observed very small regions of the microcirculation filled with two colors of Microfil. Superficially, this could be considered evidence of focal anastomoses between two vascular beds. In fact, it was always seen in association with a large feeding vessel in the vicinity which also had double filling. We believe, therefore, that these areas resulted from cross circulation between epicardial collaterals that could have been the result of temporarily unequal pressures in the two or three arms of the perfusion apparatus, or secondary to unrecognized vessel obstruction. In either case, a small number of functioning epicardial collaterals during the perfusion would allow mixing of Microfil, so that downstream it would appear as double filling of the microcirculation. Despite consistently finding larger vessels filled with two colors of Microfil in these zones, we cannot rule out the possibility that there may be some minimal cross circulation between two microvascular beds. However, we did not find obvious anatomic connections in the cardiac microcirculation, as we did in the skeletal muscle and brain. Consequently, we are confident that our observations of focally double-filled vessels are technical artifacts.

This study and the previous study in the dog were designed to reveal whether microvascular anastomoses exist in the heart. It has been assumed for many years that myocardial capillary anastomoses are present; Baroldi and Scomazzoni refer to several early twentieth century papers in which this was described, and Gross detailed this pattern in 1921. Brown and Ludwig more recently claimed that there are capillary connections in the myocardium. In both the early and contemporary studies, however, the source of the large coronary artery supplying a particular vascular bed was not identified because injection masses of only one color were perfused into the coronary artery. Without visual separation of discrete beds (such as by color, using epillumination of the Microfil perfusate), it is almost impossible to determine if regions of the microcirculation are connected. This can be illustrated by looking at just the yellow-filled vascular bed in figure 2; the density of the microcirculation alone limits any judgments about anastomoses. Had the red-filled zone also been perfused with yellow Microfil, no discrimination between the two vascular beds would be apparent. Similarly, in the studies cited above that support capillary anastomoses, the absence of any means to identify a large vessel source at the tissue level precludes making firm conclusions about connections and does not allow for a determination of double filling within the same microvessel.

If the cardiac microcirculation is discrete and organized as a true end-vessel system, then obstruction of a small arteriole near the point where it gives off a capillary arcade, but below any preformed collaterals, should lead to necrosis of small volumes of myocardium. In fact, recent studies from our laboratory have demonstrated this phenomenon. Coronary emboli-
zation of 25-μm microspheres that lodge in arterioles leads to focal areas of micronecrosis in the dog heart. The necrotic foci have a relatively uniform size range, suggesting that the coronary vasculature is organized into microcirculatory units at the tissue level. This observation further validates our findings in the present study. If the microvasculature were interconnected, one would assume that blockage of a single arteriole could not lead to necrosis of a small number of myocytes, since other anastomosing capillaries could supply oxygenated substrate. The absence of this protection demonstrates that the microvasculature is not sufficiently interconnected, if at all.

Scharrer described similar observations and conclusions more than 40 years ago, in elegant studies of the opossum cerebral microcirculation. The evolutionarily more primitive brain of the marsupial opossum is supplied by end-capillary loops with discrete perfusion fields (in contrast to the diffusely anastomosing system of placentate mammals, such as we describe in this report). When lycopodium spores were emboiled into the cerebral microcirculation, focal areas of necrosis were produced. Scharrer concluded that the cerebral vessels in this mammal were true end-arteries and that overlap of the microcirculatory bed was not sufficient to protect small regions of brain from necrosis. Our results in the canine and human heart are identical.

Our observations of discrete and unconnected microvascular beds in the canine and human heart suggest that the geographical pattern of experimental myocardial infarction determined by the microcirculatory anatomy may be applicable to the clinical situation. Creatine kinase depletion is homogeneous from the center to the lateral edge of canine myocardial infarction when tissue supplied by the nonoccluded vessel is excluded from analysis. With serial section histology, we also demonstrated that the lateral region of canine infarcts is composed of sharply demarcated interdigitating peninsulas of normal and necrotic tissue, with no evidence of a histologic border zone. More recently, we showed that the sharp boundary between normal and infarcted myocardium in the dog is determined by the microvascular supply, with only a 30-50-μm-thick zone of discordant tissue at the border. This border zone is small enough to be explained by diffusion of substrate from the patent to the occluded vascular beds.

Other studies, using different techniques, have suggested that the border zone in the experimental infarct model is relatively discrete, sharply demarcated and of insignificant dimensions. Hearse et al., who claimed that there was a border zone 8-15 mm wide representing a gradient of damage from the center to the lateral edge of an infarct, recently concluded that the lateral border was composed of a mixed population of normal and severely damaged cells. Though contrary views supporting the border zone concept remain prevalent, the discrepancy between these studies and those that suggest that the border zone is of minimal size, may represent technical limitations of the experimental methods used to define tissue at risk of necrosis within the occluded vascular bed. Since capillary blood supply is a major determinant of myocellular viability, a failure to identify the microvascular risk region may lead to erroneous conclusions.

Our findings that the coronary microcirculation in both dogs and humans is discrete and unconnected to heterologous capillary beds raises the possibility that the border region in clinical infarcts is similar to that of the experimental model. In fact, recent data from Lee et al. suggest that this may be the case. They used postmortem coronary perfusions with barium sulfate to demonstrate vascular risk regions. They showed that there was a lateral border zone of viable tissue less than 2 mm wide (mean 1.7 mm) within the occluded coronary bed. Since barium sulfate does not perfuse the microcirculation, the border zone might have been smaller than 2 mm if the capillary bed had been identified. These data, then, provide circumstantial evidence that the geometry of human myocardial infarctions is determined by the vascular anatomy, as we and others have shown in the dog.

We have demonstrated that the human myocardial microcirculation is organized into discrete capillary beds, with no evidence of heterologous capillary connections and no pronounced heterologous capillary overlap. The presence of an end-capillary loop system in the human heart suggests that there is no obvious anatomic arrangement of the microcirculation that could explain a significant ischemic border zone lateral to a myocardial infarction. For a border zone to exist, other mechanisms, such as local differences in contractility, cellular metabolism or enhanced substrate diffusion from the normal vascular bed, may have to be invoked to explain its occurrence. Studies from our laboratory and by others, however, suggest that the lateral limits of an infarct are determined by the vascular anatomy, particularly if it is identified at the microvascular level.

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