

The Role of the Coronary Microcirculation in Myocardial Recovery from Ischemia

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The aim of thrombolysis, angioplasty, and coronary artery bypass surgery is to "reperfuse" ischemic myocardium; however, reperfusion can cause further cardiac damage and compromise the coronary microcirculation. Because nutrient supply and exchange and delivery of pharmacologic agents require a patent microvasculature, the coronary microcirculation plays a major role in myocardial recovery from ischemia. It is known that ischemia-reperfusion can cause an increase in coronary permeability and microvascular plugging (No-reflow). The permeability to macromolecules is increased more than the permeability to smaller molecules. The permeability increase leads to extravasation of plasma proteins and a permeability edema. Furthermore, proteins that normally remain extravascular are now free to wash out the heart. Both microvascular effects, increased coronary permeability and No-reflow, compromise cardiac function. The degree of damage depends on the nature (No-flow versus low-flow) and length of ischemia. Unfortunately, both the increase in coronary permeability and the reduction in perfused capillarity advance with time during early reperfusion. Although the increase in permeability does not require the presence of platelets or leukocytes, it is apparent that the No-reflow response does. Mechanisms that may explain the microvascular responses to ischemia include cell swelling, damage caused by oxygen free radicals, and inflammatory responses that may or may not involve granulocytes. The permeability response may involve a calcium-mediated endothelial contraction because the macromolecular leakage that follows ischemia can be prevented by pretreating hearts with the calcium blocker nisoldipine. Protection of the coronary microcirculation should be included in any attempt to improve treatment of occlusive coronary artery disease.

INTRODUCTION

Modern man is painfully aware of the deleterious effects of myocardial ischemia. Heart attacks and stroke, which have similar etiologies, are two major killers. In most cases, the cause of organ failure is secondary to some compromise of the cardiovascular system. Angioplasty, thrombolysis, and coronary bypass surgery all attempt to remedy the vascular cause of cardiac damage. Similar methodologies are used to treat stroke. Currently, attempts are being made not only to correct the vascular problem but also to preserve or protect organ function by enhancing cell metabolism or by preventing cell necrosis. These efforts at protection are certainly worthwhile but usually do not consider a critical problem or consequence of ischemia: damage to the microcirculation.

Microvascular damage is an important consideration because it is at the level of microvessels that the principal functions of the cardiovascular system take place. In

the terminal vascular bed, blood-tissue exchange of nutrients and waste products occurs. It is also at this level that local blood flow is controlled and fluid balance is maintained [1]. If microvascular integrity is compromised irreversibly by ischemia, substrate supply may be reduced, and pharmacologic agents designed to protect cells would probably be useless, because they have no avenue to reach the target tissues.

With this introduction two questions arise: How significant is the microvascular damage due to ischemia? And, if it is important, what can be done about it? The purpose of this paper is to describe studies, largely from our laboratory, that were designed to address these questions. We have been particularly interested in preserving microvascular function in the heart [2,3,4] and more recently have performed studies aimed at attenuating the effects of ischemia on the hepatic microcirculation [5].

Initial Studies of Myocardial Ischemia-Reperfusion

To gain an understanding of the coronary microvascular damage caused by myocardial ischemia, we required a model that would reliably mimic the pathology and allow assessment of microvascular function. The literature indicated that ischemia followed by reperfusion lead often, but not always to edema and cell swelling [6], appearance of plasma proteins in cardiac lymph [7], and possible changes in microvessel ultrastructure [8,9,10,11]. These microvascular alterations occurred simultaneously with decreased metabolism and cardiac function. The degree of these deleterious changes was a function of the animal species studied, the length of ischemia, and the length of reperfusion after ischemia. Other important considerations were the type of ischemia: low-flow versus no-flow, regional versus global. Furthermore, the degree of coronary "collateralization" appeared to be an important consideration in interpreting results of ischemia-reperfusion studies [12].

Initially, we chose to study a dog model of global myocardial ischemia. Because of our interests in cardiac protection during surgery, we developed a model of cardiopulmonary bypass with separately controlled coronary perfusion and collection of coronary venous return. This model proved to be quite versatile [2,13] and was employed in other cardiac protection studies as well [14]. Because the principal function of any microcirculation is transvascular exchange, we used techniques to assess the capacity of the coronary microcirculation to exchange solutes before and after ischemia. In addition, myocardial oxygen delivery, myocardial oxygen extraction, and cardiac water content were measured to correlate with the solute exchange results.

To assess transc coronary solute exchange, we used the reference indicator technique popularized by Crone [15,16]. Measurements of the myocardial extraction of radiolabeled inulin (5,200 MW) and albumin (BSA) (69,000 MW) were made during a single pass through the coronary bed. This technique required injection of an intravascular reference tracer along with the diffusible test tracers. Because earlier studies suggested that plasma albumin leaked into the tissue space after ischemia [7,17], red cells labeled with technetium-99m were chosen as the intravascular reference tracer. Recent studies by us [18] and others [19,20] confirmed that marked extravasation of plasma protein followed ischemia. We chose to evaluate the effects of 45 minutes of global normothermic ischemia because this insult caused moderate damage with roughly 60 percent recovery of cardiac function after reperfusion. Also, there was evidence in the literature that this length of ischemia did not lead to extravasation of red cells [8]. In our initial studies, we found that roughly 50 percent of the injected inulin was extracted in a single pass as it transversed the coronary bed

[21]. This myocardial extraction was five to ten times the inulin extraction reported earlier by others in working hearts [22,23]. The larger value of inulin extraction observed in our study was caused most likely by the conditions of the experimental model. In our case, the animals were "on" cardiopulmonary bypass (CPB). This surgical procedure effectively removes the lungs from the cardiovascular system. During CPB, Pang et al. [24] and Pitt et al. [25] found increased bradykinin and prostaglandin levels in the circulating blood. These substances are normally extracted from blood as it passes through the pulmonary circulation. The elevated circulating levels of bradykinin and prostaglandins during CPB may have caused some vasodilation and an increase in coronary vascular permeability. These effects may explain the increased solute extractions that we observed under control CPB conditions [21].

In our initial ischemia-reperfusion study [13] we found that the myocardial inulin extraction decreased 38 percent during reperfusion but the albumin extraction actually increased 45 percent during the same time period. We confirmed that this curious finding was not caused by some artifactual rheologic problem and suggest that two different microvascular alterations occurred simultaneously during reperfusion. Namely, coronary vascular permeability increased with time after ischemia, despite the apparent decrease suggested by the inulin extraction data. Increased vascular permeability after ischemia may appear to be fortuitous because it increases myocardial exchange capacity, defined as the product of vascular permeability and the exchanging vascular surface area (PS product). However, a marked permeability increase leads to plasma protein leakage into the tissue space and edema [1,26]. Another deleterious consequence of increased permeability during reperfusion is that interstitial solutes or those released from damaged myocytes are not restricted from diffusing out of the tissue space into the plasma space. An example of this is seen in the elevated levels of creatine kinase (CK-MB) found in coronary sinus effluent after a myocardial infarct. CK-MB is an 80,000 MW protein that does not normally cross the cell wall of intact myocytes or intact microvasculature. Ironically, coronary venous CK-MB levels serve as an excellent clinical measure of myocardial damage from infarction or cardiac surgery [27].

In addition to increasing vascular permeability to both small and large solutes, the observed decrease in inulin extraction during reperfusion suggested ischemia-reperfusion caused a significant decrease in the exchanging vascular surface area. This effect may have been caused by vascular plugging or a No-reflow phenomena [8,9,10,11,28,29]. To assess the degree of No-reflow that follows moderate global ischemia, we [30] measured perfused capillarity in rat hearts after 30 minutes of ischemia and ten minutes of reperfusion. Perfused capillaries were marked in a manner similar to that employed earlier in skeletal muscle [31]. The results indicated that the blue dye filled 1,800–2,300 coronary capillaries/mm². No difference was observed between subendocardial and subepicardial capillarity under control conditions; however, after ischemia, we observed a marked decrease in perfused capillarity: a 70 percent decrease in subepicardial capillarity (epi) and a 90–95 percent decrease in the subendocardium (endo). The greater No-reflow in the endo samples was statistically significant compared to that in the epi. Because these hearts were beating but not ejecting, these results suggest that the endocardium was particularly vulnerable to No-reflow. The differential effect may be caused simply by the architecture of the coronary bed. With such a marked No-reflow throughout the heart during reperfusion (70–95 percent), it is not surprising that cardiac function deteriorated. Even if the myocyte were only mildly damaged by ischemia, the com-

promised nutrient delivery and exchange system during reperfusion would insure eventual cardiac necrosis. Recently Uretzky et al. [32] confirmed, using a different model, that the No-reflow phenomena after myocardial ischemia-reperfusion was more pronounced in the endocardium. Furthermore, they found that the relatively greater reduction in endocardial flow became worse as reperfusion continued. Our finding of decreased inulin extraction and the later findings of Cohen et al. [30] and Uretzky et al. [32] suggest that reperfusion makes matters worse. These findings agree with other reports of "reperfusion injury" [33,34].

To determine if cold blood cardioplegia protects the heart and coronary microcirculation from ischemia-reperfusion, we [2] performed a study in which a group of dogs was subjected to 45 minutes of global myocardial ischemia followed by reperfusion as described above. These hearts were treated, however, with a cold blood cardioplegia solution consisting of pump blood from the extracorporeal circuit cooled to 5°C with 30 meq/L potassium. Administration of cardioplegia was repeated after 15 and 30 minutes of ischemia. We found that the cold potassium cardioplegia caused some coronary vasoconstriction early during the reperfusion period [35]. The vasoconstriction was in sharp contrast to the marked vasodilation observed in the untreated group early in reperfusion. The coronary constrictor effort of the blood cardioplegia abated with time, probably because of potassium washout. After 45 minutes of reperfusion, the coronary resistances of both groups were not different from their respective control values. Furthermore, for the cold cardioplegia group, there was neither a decrease in inulin extraction nor an increase in myocardial albumin extraction, as was observed for the unprotected group. In fact, the solute exchange results for the cardioplegia group were quite similar to those seen in the earlier control group which was simply placed on CPB with no ischemic period [21].

The cardioplegia study indicated that the cold blood recipe provided excellent protection of the coronary microcirculation despite a transient vasoconstriction. Furthermore, it appears that platelets and white cells in blood cardioplegia do not induce microthrombi development. Although cold blood cardioplegia is reported to offer superior myocardial protection in comparison to crystalloid cardioplegia [36], the underlying mechanisms are not known. Our studies suggest that improved microvascular protection may be an important factor. Evidence for this suggestion is described below and comes from studies in which we visualized directly the effects of perfusate composition on coronary microvascular exchange and perfusion.

Direct Visualization of Coronary Microvascular Exchange and Perfusion

The studies described above indicated that solute delivery, solute extraction, and the semipermeable barrier characteristics of the coronary microcirculation were compromised severely after ischemia-reperfusion. Other workers report diminished transc coronary exchange after low-flow ischemia and regional myocardial ischemia and attribute the compromised exchange to reduced tissue uptake [37] or a physiologic capillary derecruitment [38]. These possibilities may be valid, but they cannot be tested with whole organ radio-tracer extraction techniques. Solute extraction from blood to tissue or vice versa is a function of blood flow, vascular permeability, exchanging vascular surface area, and heterogeneity of perfusion. Using whole organ radio-tracer techniques, one can only make a good guess as to the actual cause(s) of an observed change in tracer extraction.

To determine the mechanisms underlying the microvascular damage caused by ischemia, a simpler, more direct model was needed. Toward this end, we developed

a model for direct visualization of the coronary microcirculation. Rat hearts were initially perfused *in situ* with a Krebs blood solution high in potassium and then isolated and placed on a special microscope stage. A conjugate of fluorescent albumin (FITC-BSA) [39] was added to the perfusate and the epicardial microcirculation viewed by illuminating the fluorescent plasma with a high-quality fluorescence microscope (Zeiss). In this manner, we were able to view the epicardial microcirculation of the arrested heart with remarkable resolution. In preliminary studies we observed that the fluorescent plasma protein (FITC-BSA) remained largely intravascular only when the Krebs perfusate contained both red cells and albumin. To quantify these effects, we developed a videodensitometric technique to measure FITC-BSA extravasation [40].

Our initial direct visualization studies, in which the composition of the coronary perfusate was varied, lead to the conclusion that coronary perfusion with a Krebs-like solution causes a striking increase in coronary vascular permeability to macromolecules. The observed protein effect and cell effect suggested that both blood constituents had direct effects on the vascular wall [3]. The protein effect had been demonstrated in other vascular beds [41] and in the heart for small solute exchange [42] but had not been previously demonstrated for macromolecular exchange in the heart. We were concerned that these observations were artifactual and tested the possibility that the increased vascular permeability with Krebs perfusion was caused simply by hypoxia. A group of hearts was perfused with Fluosol-DA (Green Cross, Inc.), which is an experimental blood substitute with increased oxygen-carrying capacity and contains colloid (HES). We found that hearts perfused with 100 percent Fluosol-DA had much more FITC-BSA extravasation compared to those perfused with the Krebs albumin-red cell solution [43]. A follow-up study indicated that adding red cells and albumin to Fluosol-DA perfluorocarbons (PFC) reduced the FITC-BSA leakage to a level similar to that observed with the Krebs-BSA-RBC perfusate [44]. Thus the transc coronary macromolecular leakage observed with pure Fluosol-DA was caused by the absence of albumin and red cells rather than any toxic effects of Fluosol perfluorocarbons.

The increase in coronary vascular permeability to FITC-BSA caused by Krebs perfusion was striking and occurred rather quickly. These observations may explain the superior cardiac protection of blood versus crystalloid cardioplegia. Crystalloid cardioplegia may increase coronary vascular permeability during cardiopulmonary bypass because these solutions usually do not contain albumin and never contain blood cells. Furthermore, because our earlier results indicated elevated coronary vascular permeability from CPB alone, an additional increase in vascular permeability would make these hearts particularly vulnerable. Cold blood cardioplegia may protect vascular integrity by both its protein and cell effects. Such protection would reduce the potential for a permeability edema [26] and perhaps minimize washout of critical tissue solutes and enzymes such as CK-MB.

The direct visualization technique combined with the control afforded an isolated perfused heart model lead to other interesting observations of the nature of coronary microvascular damage that follows ischemia-reperfusion. In a study reported recently [18], we found that ischemia-reperfusion led to marked transc coronary FITC-BSA extravasation. These direct observations substantiated our earlier indirect measure of increased albumin extraction [2]. The direct visualization experiments allowed a continual view of transc coronary FITC-BSA extravasation during reperfusion. Early in reperfusion, no noticeable FITC-BSA leakage was ob-

served; however, pronounced leakage was seen after ten minutes, and the leakage appeared worse after 20 minutes of reperfusion. The increased extravasation with time indicated that the extravascular FITC-BSA accumulation in the tissue space may be caused by an ischemia-induced increase in permeability which was further increased by reperfusion.

The second finding from the direct visualization study of ischemia-reperfusion was that we did not observe a No-reflow phenomenon during reperfusion. Perfused capillarity was determined by counting the number of red cell perfused capillaries crossing a 200 μm raster line positioned on the video monitor. We found no significant decrease in this measure of capillarity during reperfusion [18]. This result was in sharp contrast to our earlier findings of a 70 percent decrease in perfused capillarity during reperfusion [30]. It occurred to us that the perfusate used in the later study did not contain many platelets or white cells (about 0.1 percent of normal for each). On the other hand, in our earlier study, in which a marked No-reflow was demonstrated, the hearts were perfused with whole blood from a support animal [30]. These results suggest that platelets and/or white cells play a significant part in the No-reflow phenomenon that follows myocardial ischemia. Recently, we performed direct visualization, ischemia-reperfusion studies with platelets and white cells in the K(2)RBC perfusate and did observe No-reflow. In several experiments we also saw a more pronounced plasma protein leakage. These findings tend to support the work of Schmid-Schoenbein and Engler [45] who found leukocyte aggregation in coronary microvessels after ischemia. Other hypotheses to explain the No-reflow phenomena include: endothelial cell swelling [8], microvascular compression caused by edema or myocardial contracture [29,46], and coronary vasospasm [11]. Our current feeling is that microthrombi development is the principal cause.

To examine the role of calcium in the observed increase in coronary vascular permeability after ischemia, we treated a group of rat hearts with a calcium blocker (nisoldipine, Miles Laboratories, 1 $\mu\text{g}/\text{minute}$) for five minutes prior to ischemia. The results were striking. Nisoldipine pretreatment prevented the FITC-BSA extravasation caused by ischemia reperfusion. In contrast to the 76 percent increase in transcoronary albumin (FITC-BSA) leakage ($p < 0.01$) measured during reperfusion in the untreated group, we measured only a 3 percent (N.S.) increase in the nisoldipine group [4]. These results implicate a calcium-mediated mechanism in ischemia-induced vascular permeability alterations. One attractive speculation is that the microvascular exchange vessels regulate large solute permeability by forming gaps between endothelial cells. Such a hypothesis was proposed earlier by Majno et al. [47] to explain the action of histamine on microvascular permeability. It appears that vascular endothelial microfilaments do contain contractile proteins [48]. If endothelium can contract, and if it can change its shape, then interendothelial gaps could indeed form, permitting macromolecular exchange.

The exciting extension of the histamine response theory is that calcium influx into endothelia may mediate this response. Calcium mediates contraction in cardiac, skeletal, and smooth muscle. It may mediate endothelial contraction as well. It is well established that, in ischemia, the myocyte cell wall permeability increases. The increase in permeability leads to intracellular calcium accumulation [49]. Calcium blockers are thought to protect the heart from damage by preventing this calcium influx. Just as occurs in the myocyte, calcium may enter vascular endothelium following ischemia-reperfusion, either because of direct ischemic damage or reperfusion injury caused by oxygen free radical formation [50]. In either case, this influx may cause endothelial microfilament contraction and induce gap formation between

vascular endothelium. This effect would alter microvascular exchange at the capillary and venular level. It may also effect endothelium-mediated smooth muscle contraction at the resistance vessel level [51,52]. Nisoldipine may have blocked calcium uptake into endothelium and prevented gap formation. Further studies are needed to confirm or reject this hypothesis, but one result is clear. In K(2)RBC perfused hearts, the calcium blocker nisoldipine did indeed prevent the coronary permeability increase caused by ischemia-reperfusion. The underlying mechanisms remain unexplained.

The studies described above delineate the nature of coronary microvascular damage from ischemia and offer some hope that these deleterious effects can be prevented or, at least, attenuated with pharmacologic agents. The truly exciting challenge, which we hope to pursue, is to rank the underlying causes of microvascular damage caused by ischemia and attempt to treat the worst offenders. Protecting the heart from the deleterious effects of coronary disease and during cardiac surgery is an important endeavor. Protecting the microcirculation of the heart and other organs from ischemic damage will greatly facilitate overall organ recovery. Furthermore, efforts aimed at preserving transplantable organs, such as the liver, the kidneys, and the heart, should consider the critical role played by the microcirculation in maintaining normal function.

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