Resting myocardial flow in hibernating myocardium: validating animal models of human pathophysiology

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CHRONIC REVERSIBLE CONTRACTILE DYSFUNCTION is frequently identified in the evaluation of patients with coronary artery disease. There is intense clinical interest in this area because it impacts directly on clinical decision making, and it has been the subject of several recent reviews (6, 23, 25, 46, 48). Nevertheless, until recently, basic understanding of physiological mechanisms has lagged far behind clinical descriptions because of the lack of appropriate animal models of the human disease. This contrasts with myocardial stunning (i.e., the transient dysfunction observed despite normal resting perfusion following acute ischemia), where data from animal models preceded clinical studies demonstrating its importance in humans (2). A particular controversy at present relates to whether chronic contractile dysfunction simply reflects repetitive stunning or whether the heart has the intrinsic capability to alter its phenotype in response to repetitive episodes of ischemia in a way that reduces its vulnerability to ischemia and results in "hibernating myocardium." At the center of the current controversy is whether resting myocardial perfusion in viable dysfunctional myocardium is normal or reduced. Available clinical studies summarizing quantitative measurements of perfusion in patients and direct measurements in several recently developed chronic animal models of viable chronically dysfunctional myocardium are discussed below.

HISTORICAL PERSPECTIVE OF THE CLINICAL PROBLEM

There has been considerable interest in prospectively identifying patients with chronic left ventricular contractile dysfunction (with as well as without symptoms of heart failure) that improves following percutaneous coronary intervention or coronary bypass surgery. Reversible dyssynergy was identified as an important clinical entity on the basis of observations from clinical trials demonstrating improved regional and global function in selected patients undergoing saphenous vein bypass revascularization (38, 39). Reversible dyssynergy could sometimes be identified prospectively by demonstrating an acute improvement in contractile function after nitroglycerin and postextrasystolic potentiation (4). A rather surprising observation was that dysfunctional segments demonstrating recruitable functional reserve frequently had evidence of reduced regional uptake of single photon tracers such as thallium, suggesting that resting myocardial perfusion was reduced. Whereas these defects had traditionally been interpreted to be indicative of either acute ischemia or myocardial scar, objective evidence or subjective signs of myocardial ischemia were lacking, and follow-up of patients after revascularization confirmed an improvement in contractile function. These clinical observations gave rise to the notion that viable dysfunctional areas were relatively hypoperfused at rest compared with normal remote regions of the same heart, and this situation was subsequently termed "hibernating myocardium" (4, 38). Nevertheless, skepticism as to how this could develop without signs of ischemia and doubt about whether such a state could be chronic without resulting in necrosis has given rise to the controversy of whether resting perfusion is indeed reduced in such patients.

RESTING PERFUSION IN VIABLE CHRONICALLY DYSFUNCTIONAL MYOCARDIUM IN HUMANS

A number of investigational techniques are available to quantify resting myocardial perfusion in humans. Using inert gas washout, Arani et al. (1) measured absolute resting perfusion in a group of patients with total left anterior descending artery (LAD) occlusion and collateral-dependent myocardium. They found reduced resting flow in the presence of varying degrees of anterior dysfunction. Resting flow values averaged 0.38–0.51 ml·min⁻¹·g⁻¹ and, when corrected for double product, they were below the 95% confidence intervals for normals that averaged 0.76 ml·min⁻¹·g⁻¹. Resting flow measurements in viable chronically dysfunctional myocardium have also been derived from dynamic analysis of [¹¹C]ammonia and [¹⁵O]water using positron emission tomography (PET). Values in relation to those measured in normally perfused remote regions are summarized in Table 1. A similar analysis has also recently been reported by Heusch (23). The first group are from studies examining viable chronically dysfunctional myocardium in patients with total coronary occlusions and collateral-dependent myocardium without a history or clinical evidence of previous myocardial...
infarction (30, 31, 41, 47). The second group includes patients with and without myocardial infarction in whom functional improvement was documented following coronary revascularization (13, 15, 20, 22, 32). The third group includes patients in whom viability was identified by an 18F-2-deoxyglucose (FDG)/flow mismatch pattern (5, 14, 33, 44). Even when grouped by studies excluding infarction versus those in which patients with an infarction were included, resting flow in viable chronically dysfunctional myocardium was reduced. There were similar differences in all of the subgroups, with flow in dysfunctional regions averaging 0.71 ml·min⁻¹·g⁻¹ versus 0.88 ml·min⁻¹·g⁻¹ in remote regions of the same heart (i.e., a 20% difference in full-thickness relative flow at rest). The difference in perfusion between dysfunctional and remote regions was statistically significant in 10 of the 13 studies.

Whereas some have speculated that the relative difference in resting flow is secondary to an increase in perfusion to remote zones (reflecting increased demand possibly due to compensatory hyperkinesis) (47), the remote zone values from the pooled studies (0.88 ml·min⁻¹·g⁻¹) are slightly lower than values from 14 studies quantifying resting perfusion by PET in normal volunteers that averaged 0.92 ml·min⁻¹·g⁻¹ (6). Others have argued that the flow values in hibernating myocardium are within the “normal range” and therefore not significantly reduced if one compares patients with dysfunctional myocardium to normal controls (6). The data presented in Table 1 show that the mean value of flow in dysfunctional regions is about one standard deviation below values in the remote region. An estimation of sample size (80% power to detect a difference at the P < 0.05 level) using the standard deviation of flow measurements in normal patients (0.22 ml·min⁻¹·g⁻¹) indicates that at least 19 normal and 19 dysfunctional patients would need to be studied to demonstrate a significant difference in flow in separate groups of patients. Finally, some have argued that reduced tracer activity could be due to partial volume effects reflecting severely reduced regional wall thickening in dysfunctional compared with normal regions (i.e., the time-averaged wall thickness in dysfunctional regions is lower) (6, 48). It is, however, then difficult to explain normal flow in the three other patient studies with dysfunction that was as severe as that in patients where resting flow was reduced, and the inert gas washout measurements of Arani et al. (1) circumvent this potential problem.

Thus PET measurements of resting perfusion demonstrate a modest but significant reduction in resting flow in hibernating myocardium, which is fairly similar among each of the subgroups summarized in Table 1. The paired comparison of flow in dysfunctional and remote regions has statistically more power than comparing groups of patients with separate populations of normal controls. Furthermore, evaluating relative changes is the conventional way that perfusion imaging is applied to the clinical management of individual patients (i.e., each patient serves as his own control). It is apparent, however, that there is a population of patients in whom chronic dysfunction exists with normal resting perfusion, raising the possibility that they represent one end of a spectrum of physiological abnormalities.
RESTING FLOW IN ANIMAL MODELS OF VIABLE CHRONICALLY DYSFUNCTIONAL MYOCARDIUM

Whereas PET and other quantitative imaging modalities have advanced our understanding of flow and metabolism in patients with hibernating myocardium, basic research advances have been delayed because of the lack of suitable animal models in which the long-term chronic adaptations to ischemia can be reproduced and studied in the laboratory. Available measurements of flow and function from studies employing subacute (≥24 h) and chronic (>1 mo) stenosis animal models are summarized in Table 2. Subendocardial and transmural perfusion in dysfunctional versus normal remote regions is tabulated as well as the ratio between the two.

Initially, the most widely used animal model examined the effects of acute steady-state ischemia studied over a period of hours as originally described by Matsuzaki et al. (34). The early adjustments to ischemia have been previously reviewed (7, 23, 40). Models of prolonged perfusion-contraction matching or “short-term hibernation” have provided insight into the initial adaptive response of the heart to acute ischemia, indicating that it can be maintained for at least several hours without development of significant necrosis. A number of laboratories have demonstrated that prolonged moderate ischemia results in a new balance between a reduced blood supply and a reduced level of function. This can be maintained for a period of hours without developing necrosis through reductions in oxygen consumption and ATP utilization. After perfusion is reestablished, function remains reduced. Whereas flow returns to normal, consistent with myocardial stunning, there is evidence that myocardial oxygen consumption continues to be reduced consistent with a downregulation in energy utilization (43).

More recently, the duration of ischemia has been extended to 24 h (10, 26) and 1 wk (11) in an attempt to produce viable chronically dysfunctional myocardium (Table 2). This results in regional dysfunction and, by experimental design, a stenosis-induced reduction in flow. Nevertheless, a major problem with this approach is that it results in irreversible injury that produces patchy necrosis visible by 2,3,5-triphenyltetrazolium chloride (TTC) in some animals (10, 11) and focal necrosis by light microscopy in most (26). Whether this is an experimental model of hibernating myocardium or prolonged perfusion-contraction matching similar to acute nontransmural ischemia remains unknown, because it is difficult to identify the relative roles of reversible and irreversible injury.

From a clinical perspective, it is unlikely that the majority of patients develop chronic hibernating myocardium in such a fashion, with the exception of patients who acutely present with prolonged myocardial ischemia in the absence of infarction. This experimental model also appears to have fueled the controversy regarding reductions in resting flow, because many debate whether such a tenuous state of acute ischemia and exhausted coronary flow reserve can be maintained for more than a period of several hours, and the animal studies have consistently demonstrated some degree of myocardial necrosis. Finally, studies examining the temporal progression of dysfunction in chronic stenosis models that limit flow reserve suggest a fundamentally different concept, with reduced flow the result rather than cause of the chronic contractile dysfunction (9, 17, 19).

Chronic stenoses that reduce coronary flow reserve appear to be more applicable to understanding the factors governing the development of viable dysfunctional myocardium. Liedtke and colleagues (27) proposed...
posed that dysfunctional myocardium could be produced over several days by an acute physiologically significant stenosis that reduced flow reserve but not resting flow. Microsphere measurements after 4 days showed dysfunction with normal resting flow consistent with chronic stunning. When the model was maintained for 1 wk, a number of animals developed pathological and TTC evidence of infarction but, when excluded, there was still a significant reduction in function (3).

Another approach has been to simulate a chronic progressive stenosis by using an Ameroid occluder. The disadvantage of this approach is that the rate and extent of progression of the Ameroid stenosis and the rate of collateral development are unpredictable. Furthermore, total occlusion usually develops rapidly (2–4 wk from the time of instrumentation), limiting the time available for collateral growth to reach a point where it is sufficient to prevent infarction (a particularly significant problem in the pig). Nevertheless, Canty and Klocke (9) demonstrated that viable chronically dysfunctional myocardium could be produced in dogs using an Ameroid occluder and surgically ligating epicardial collaterals at the time of instrumentation (12). Serial measurements showed that function was initially reduced without any relative difference in perfusion. Interestingly, 1 wk after the Ameroid occluded, relative function improved slightly but was then accompanied by a reduction in subendocardial perfusion compared with remote myocardium, suggesting the development of hibernating myocardium. This subsequently normalized along with function as collaterals in the dog matured after 6–7 wk. Canty and Klocke (9) proposed that a state of chronic stunning was followed by a transient state of hibernation. Shen and Vatner (42) subsequently reproduced a similar state of chronic stunning using an Ameroid in the pig. They also found function to reach a nadir at a time when resting flow was normal. Whereas function improved as the Ameroid stenosis progressed and stimulated collateral development, they did not have any delayed flow measurements until the end of their study (32 days), when it was insignificantly reduced and patchy tissue necrosis was frequently present. More recently, Firoozan et al. (19) have attempted to produce viable dysfunctional myocardium using multiple left coronary Ameroid occluders in the dog. With this approach, they also demonstrated that dysfunction occurred early after Ameroid instrumentation and without reductions in resting flow consistent with chronic stunning. At the terminal study, however, there was a heterogeneous response. Some regions had depressed function with reduced flow, whereas others had depressed function with normal resting perfusion. Thus serial measurements in animals instrumented with Ameroid occluders demonstrate that contractile dysfunction is initially associated with normal resting flow. Two of the three studies demonstrate that this can be followed by a state where dysfunctional myocardium develops reduced resting flow. This suggests a temporal progression from chronic stunning to chronic hibernation in animals instrumented with a chronic stenosis.

A more long-term model of viable dysfunctional myocardium in the absence of infarction has been produced using a fixed LAD stenosis in the pig. This has been reproduced in three laboratories with studies conducted over periods of up to 8 mo (18, 35, 37). Millard (36) originally described and used this approach to stimulate collateral formation in domestic swine, and it was subsequently modified and adapted by Mills et al. (37). They found that pigs instrumented with a chronic LAD stenosis developed a 25% reduction in resting perfusion compared with remote normally perfused myocardium. Interestingly, whereas they did not assess regional function, regional oxygen consumption was reduced in comparison to historical controls. Fallavollita et al. (18) subsequently implemented this approach and demonstrated severe anterior hypokinesis with a 13% reduction in resting flow in full-thickness transmural samples. Subendocardial flow was reduced by 25% in comparison to remote zones. They concluded that there may be transmural variations in adaptations to a chronic stenosis, because the increase in the uptake of FDG was most pronounced in the subendocardium. Finally, McFalls et al. (35) implemented this model and found that flow by PET was reduced by 34% in comparison to remote zone values. Thus the fixed LAD stenosis model in pigs consistently demonstrates dysfunction with reduced resting myocardial perfusion that is of a magnitude similar to humans with hibernating myocardium in the absence of infarction (Table 1).

We (17) also examined animals at earlier time points to elucidate the temporal progression of physiological abnormalities in pigs instrumented with a fixed LAD stenosis. Pigs studied as early as 1 mo had resting dysfunction that worsened at 2 mo. Interestingly, resting perfusion at both time points was normal, consistent with a state of chronic stunning. Comparison with animals studied after 3–4 mo in which dysfunction was associated with reduced resting flow demonstrated a progression in abnormalities. Resting dysfunction with normal resting perfusion was present at 1 mo and was followed by increased FDG uptake at 2 mo. Hibernating myocardium with reduced resting flow and increased FDG uptake developed after 3–4 mo. In addition, a recent study has demonstrated that the progression to a state of reduced resting flow can be accelerated in this model by restricting source collateral flow with a second stenosis on the circumflex artery (16). The ability to accelerate the development of hibernating myocardium supports the hypothesis that variability in producing reductions in resting perfusion may reflect differences in the physiological significance of coronary stenoses and collateral vasodilator reserve.

CHRONIC STUNNING VERSUS CHRONIC HIBERNATION: CLINICAL SIGNIFICANCE

Data regarding the natural history of viable dysfunctional myocardium in humans are necessary to elucidate the potential relation between these two conditions. In lieu of this, the experimental models
reproducing chronically stunned and hibernating myocardium and studies examining the temporal progression of these abnormalities provide some insight. Studies examining the effects of an acute reduction in coronary flow reserve (27), an Amoroid occluder (9, 19, 42), and a fixed LAD stenosis (17) all demonstrate an early period in which dysfunction is associated with normal resting perfusion and is consistent with the concept of chronic stunning. With time and probably increases in the physiological significance of the chronic stenosis, there is a progression to dysfunction with reduced resting flow consistent with hibernating myocardium (9, 16, 18, 19, 35, 37). Thus a chronic stenosis may progress to a point where the frequency of ischemia (and the associated posts ischemic stunning) induce regional alterations in the myocyte (29, 46). This may be a response to limit imbalances between a limited supply and regional demand (8). In this context, hibernating myocardium could be viewed as an adaptive state that results from the injury-repair cycles associated with chronic myocardial stunning.

Data from humans and experimental animals with viable dysfunctional myocardium demonstrate that resting coronary flow can be reduced or normal. This variability among patients was originally reported in the mid 1980s by Tillisch et al. (45) in a seminal study that demonstrated the utility of PET to predict functional recovery. Function recovered when FDG uptake was preserved or increased in dysfunctional regions, yet resting flow could be normal or depressed. Normally perfused regions of the heart should, by definition, never have a significant component of myocardial scar that has a lower resting flow per gram of tissue compared with cardiac muscle (32). Thus viability of such chronically stunned regions is never a clinical problem. The clinical problem one faces is in evaluating regions with dysfunction that have depressed flow. In these patients, viability studies are necessary to distinguish hibernating myocardium from infarction. The results of these studies can have a significant impact on subsequent patient management, particularly when global left ventricular function is severely depressed (21).

Aside from distinguishing viable from infarcted myocardium when resting flow is reduced, what is the clinical importance of distinguishing these two entities? There is considerable variability in the recovery of dysfunctional myocardium among available studies, with some showing normalization as early as hours and others showing a time course that spans several months (6, 23, 25). Because acute stunning without infarction recovers within a period of hours (2), one might anticipate more rapid recovery of function in patients with normal resting perfusion. In contrast, hibernating areas with reduced resting flow appear to have more prominent phenotypic changes that allow myocytes to remain viable in a setting where they are subjected to frequent repetitive ischemia (29). These adaptive changes may also involve molecular mechanisms that affect the contractile function of the heart and take a considerable period of time to normalize after revascu-

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