Translational Physiology: Porcine models of human coronary artery disease: implications for preclinical trials of therapeutic angiogenesis

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Hughes, G. Chad, Mark J. Post, Michael Simons, and Brian H. Annex. Translational Physiology: Porcine models of human coronary artery disease: implications for preclinical trials of therapeutic angiogenesis. J Appl Physiol 94: 1689–1701, 2003; 10.1152/japplphysiol.00465.2002.—“Therapeutic angiogenesis” describes an emerging field of cardiovascular medicine whereby new blood vessels are induced to grow to supply oxygen and nutrients to ischemic cardiac or skeletal muscle. Various methods of producing therapeutic angiogenesis have been employed, including mechanical means, gene therapy, and the use of growth factors, among others. The use of appropriate large-animal models is essential if these therapies are to be critically evaluated in a preclinical setting before their use in humans, yet little has been written comparing the various available models. Over the past decade, swine have been increasingly used in studies of chronic ischemia because of their numerous similarities to humans, including minimal preexisting coronary collaterals as well as similar coronary anatomy and physiology. Consequently, this review describes the most commonly used swine models of chronic myocardial ischemia with special attention to regional myocardial blood flow and function and critically evaluates the strengths and weaknesses of each model in terms of utility for preclinical trials of angiogenic therapies.

CORONARY ARTERY DISEASE (CAD) continues to be the leading cause of mortality in the industrialized world, with over 12 million Americans alive today with a history of angina pectoris, myocardial infarction, or both (3). Despite advances in pharmacological therapies as well as catheter-based and surgical revascularization, a significant number of these patients have diffuse coronary disease, small distal vessels, or other comorbidities that make them poor candidates for traditional methods of treatment. This number may represent as many as 12% of all patients with symptomatic coronary disease (59). In addition, as the population ages, the proportion of patients ineligible for these therapies may increase. Consequently, alternative means of improving blood flow to the heart, i.e., therapeutic angiogenesis, may take on a larger role in the treatment of CAD. Although mechanical means such as transmyocardial laser revascularization (38), angiogenic peptides including the fibroblast growth factors (77) and vascular endothelial growth factors (33), and gene therapy (84) have been used clinically, none has yet proven ideal. Consequently, further preclinical study of existing as well as novel therapies is necessary to continue to advance the field. Such preclinical studies require robust large-animal models that allow assessment of both the safety and efficacy of various therapies with predictive value for clinical study. To date, little has been written comparing the various models of chronic ischemia available for use (89), and no review has focused solely on chronic in vivo large-animal models. This review describes those models most commonly employed in large-animal studies of chronic myocardial ischemia, with a focus specifically on porcine models because of their increasing use in recent years, and critically evaluates the strengths and weaknesses of each in terms of their utility for preclinical trials of angiogenic therapies.

WHAT ANIMAL MODELS SHOULD REPRODUCE: REFRACTORY ANGINA PECTORIS AND END-STAGE CAD

Clinical trials of therapeutic angiogenesis have generally included patients with refractory angina pecto-
ris and so-called “end-stage” CAD (75). This term refers to patients with the persistence of severe anginal symptoms (Canadian Cardiovascular Society Class III and IV) despite maximal conventional antianginal combination therapy and coronary atherosclerosis not amenable to revascularization by percutaneous means or surgical bypass. The overwhelming majority of these patients have multivessel coronary disease and have undergone prior revascularization procedures (75). However, patients eligible for these alternative therapies generally have a relatively large amount of viable myocardium and only moderately impaired left ventricular function. Consequently, heart transplantation is not an option for this patient population (75). Patients with large areas of prior myocardial infarction and its attendant necrosis and scar formation are excluded because these changes are not reversible with improvements in myocardial perfusion (19). Rather, patients should have ischemic yet viable myocardium as demonstrated by positron emission tomography, thallium or technetium-sestamibi scintigraphy, or dobutamine echocardiography. The latter situations are reversible with improvements in myocardial blood flow (19).

Because of the heterogeneity of coronary artery disease and the unpredictability of collateral development, no two patients with symptomatic coronary disease will have the same pathophysiology or clinical features (61). In general, however, patients will manifest with ischemia, defined as a lack of oxygen at the cellular level caused by inadequate coronary flow (61). Patients eligible for proangiogenic therapies are frequently considered to have areas of “hibernating” myocardium (87), although in reality many will have some combination of the various recognized clinical ischemic syndromes such as effort ischemia, chronic myocardial stunning, and chronic hibernation.

Chronically stunned myocardium describes regions of the heart with persistent dysfunction at rest despite normal basal perfusion (88). This condition of persistent postischemic dysfunction is felt to result from repetitive episodes of stress-induced ischemia in myocardial regions with impaired coronary flow reserve. This differs from chronic myocardial hibernation, which refers to myocardial regions with persistent dysfunction but which are hypoperfused at rest (88). Although these ischemic syndromes appear distinct on paper, there is likely much overlap between them in the clinical setting. Common to both conditions are characteristic structural alterations (Fig. 1) affecting both the cardiomyocytes and extracellular matrix, including loss of contractile material, glycogen accumulation, numerous small mitochondria, irregular nuclei, and cytoskeletal alterations, as well as increased collagen, fibronectin, and structural proteins, among others (88).

A recent study has demonstrated that, in swine with high-grade coronary stenoses and dysfunctional yet viable myocardium, chronic stunning and hibernation appear to coexist, with approximately two-thirds of the dysfunctional segments within the distribution of the stenotic epicardial coronary artery being hypoperfused at rest (hibernating) and one-third normally perfused (stunned) (43). Similar findings have been made in a

![Fig. 1. A: electron microscopy (original magnification ×900) of biopsy from chronic hibernating myocardium in a miniswine with an experimentally produced high-grade proximal left circumflex coronary artery stenosis. Note loss of contractile elements within viable cardiomyocytes (arrowheads). These changes are most prominent in the perinuclear area. B: on higher power (original magnification ×7,100), large areas of glycogen accumulation (G) are visible within the areas of sarcomere loss along with numerous small mitochondria (M). N, nucleus. C: nonischemic anteroseptal region demonstrating normal ultrastructure (original magnification ×900). Reproduced from Ref. 82 with permission.](http://jap.physiology.org/10.1152/jappl.00501.2002)
porcine aimeroid constrictor model (65). Fallavollita and Canty (25) have put forth the theory of a transition from stunning to hibernation over time in viable, chronically dysfunctional myocardium. Although the exact contribution of chronic stunning and hibernation to the clinically observed syndrome in any particular patient may not be discernible, differentiation between the two is probably not clinically relevant because the treatment for both conditions involves improving myocardial perfusion, which if done in a timely manner will result in improvement in left ventricular function (37).

One important consideration is that there appears to be a spectrum in terms of recovery of function after revascularization under these conditions (61). As noted above, there are structural changes that occur in chronically dysfunctional myocardium, and these alterations are felt to affect the ability of the myocardium to recover function after revascularization (88). Elsässer and colleagues (22) have described a self-perpetuating “vicious cycle” in hibernating myocardium whereby a regional inflammatory reaction in the ischemic territories leads to progressive fibrosis and cellular degeneration. The importance of the progressive nature of these phenotypic changes is that patients with more advanced cardiomyocyte deterioration have less return of function after revascularization than those with less advanced changes (23, 32, 76). Because the therapeutic angiogenesis trials to date have generally included “no option” patients with severe inoperable CAD, one should not be surprised that improvements in ventricular function seen in these trials have been insignificant.

LARGE-ANIMAL MODELS PRESENTLY IN USE

Maxwell et al. (55) in 1987 demonstrated the wide spectrum of collateral flow that exists between various mammalian species (Fig. 2). This study demonstrated that the dog, which for many decades has been the most commonly used species in studies of myocardial ischemia, has a variable and often substantial collateral circulation network. These preformed collaterals are capable of providing up to 40% of normal flow to the perfusion bed of an acutely occluded epicardial coronary artery (31). Consequently, most recent large-animal models of chronic ischemia have utilized swine because their coronary anatomy, with minimal preexisting coronary collateral vessels, is similar to that of humans (31, 55, 74). In addition, their cardiac physiology, with the distribution of the coronary artery blood supply, including a typically right-dominant coronary system, and cardiac conduction system are very similar to humans (83). Likewise, the heart size-to-body weight ratio (0.005) for the typical 30-kg pig used in most laboratory studies is identical to that of humans (36). Finally, the swine heart is similar to that of humans from a metabolic standpoint as well, relying predominantly on nonesterified fatty acids as the preferred substrate under normal conditions, accounting for up to 80% of myocardial energy production (1). During sublethal myocardial ischemia, the β-oxidation of fatty acids diminishes concomitant with increased glucose extraction (Randle’s cycle) (1), a property we and others have demonstrated using positron emission tomography (PET) imaging of the chronically ischemic porcine heart (43). These anatomic and physiological factors, which have been reviewed in detail elsewhere (36), become critical in selecting an animal model for the study of therapeutic angiogenesis. Consequently, this review will focus on the most commonly used swine models of chronic, reversible myocardial ischemia. These studies have generally utilized one of three methods for producing stenosis or occlusion of an epicardial coronary artery: an aimeroid constrictor, fixed stenosis, or hydraulic occluder. Other models, such as the repetitive coronary occlusion model (46, 54a, 91), that have been used exclusively in dogs will not be discussed. Because the ultimate goal of proangiogenic therapies is improved myocardial perfusion and function, special attention will be given to regional myocardial blood flow (MBF) and function in the various porcine models.

Aimeroid constrictor model. The most widely used porcine model of chronic ischemia has been the aimeroid constrictor (24, 45, 60, 66), which has been utilized in preclinical studies of numerous proangiogenic therapies including recombinant vascular endothelial growth factor (69) and basic fibroblast growth factor (47, 68), autologous bone marrow (28), and gene therapy (54), as well as transmyocardial laser revascularization (35, 70). Originally described by Litvak and colleagues in 1957 (52), these constrictors are constructed of the hygroscopic material casein encased within a steel sleeve. When the device is implanted around an artery, the constrictor absorbs water and swells, compressing the artery and producing total coronary occlusion over a period of 14–30 or more days (24, 45), a period that may be prolonged by coating the aimeroid with petrolatum, thus slowing the absorption of water by the occluder (90). However, as discussed in a recent review (87), although aimeroid constrictors cause external arterial compression as their cross-sec-
tional diameter diminishes, they also cause mechanical trauma that may lead to endothelial damage, platelet aggregation, and thrombus formation, as well as potentially inciting a foreign body reaction with local scar formation. Consequently, the common perception that these constrictors cause gradual coronary occlusion may be an oversimplification (87).

Most commonly, these constrictors have been placed on the left circumflex (LCx) coronary artery, which is the smallest of the three coronary vessels in swine, supplying ~20% of the left ventricular myocardium (36, 66). In addition, the LCx region of myocardium in pigs has a greater innate collateral circulation than regions of the left ventricle supplied by the left anterior descending or right coronary arteries (92). However, despite this there is still a significant incidence of sudden cardiac death due to ventricular fibrillation or massive myocardial infarction in swine instrumented with LCx occluders, which averages nearly 30% in those studies reporting mortality rates (28, 68, 69). The vast majority of these ischemia-related sudden deaths occur during the period of ameroid closure. As noted above, swine, unlike dogs, have minimal preexisting coronary collaterals. Consequently, they tolerate acute coronary occlusion poorly, with large areas of infarction and death typically resulting (29, 92). Acute LCx occlusion in the pig results in infarction of ~75% of the area at risk and a mortality rate of 35% compared with 50 and 13%, respectively, in dogs (94). However, with more gradual occlusion in the pig, as often occurs after ameroid constrictor placement, a collateral circulation develops that is able to prevent or minimize myocardial infarction in many instances (74, 92). Nonetheless, likely as a result of the nonuniform rates of ameroid closure in vivo (45), there is a large variation in the reported percent infarction of the area at risk. O’Konski and colleagues (60) studied swine with ameroid constrictors placed around the LCx coronary artery and found an average infarct size of 37 ± 36% of the area at risk ~3 wk after ameroid placement. The majority of infarction occurred in the subendocardial region. As indicated by the standard deviation, there was a large variation in infarct size between animals, with a range of ~5–100% observed. There was a trend toward less infarction (17 ± 6% area at risk) in a subgroup of animals receiving aspirin during the course of the experiment. A follow-up study from this same laboratory reported a 5 ± 1% infarct of the LCx area at risk (66), which the authors attributed to less manipulation of the coronary artery. Other studies have reported infarct rates of 6–13% of the LCx area at risk (67, 85, 93).

Among the models presently in use, the ameroid constrictor has been the most extensively studied, and much of our present knowledge regarding the development of the coronary collateral circulation has been derived from studies using this model (29, 60, 66, 67, 74, 85, 92–94). Consequently, regional myocardial blood flow and function in the distribution of the ameroid occluded LCx artery have been well characterized. O’Konski and colleagues (60), using radioactive microspheres (the gold-standard for measurement of regional myocardial blood flow in experimental studies; Refs. 34, 87), demonstrated regional transmural MBF of the LCx distribution 3 wk after ameroid placement to be no different from that within the nonischemic regions of the left ventricle. However, regional transmural MBF in the LCx region was significantly reduced (42% control) during exercise-induced stress, indicating impaired coronary flow reserve. Similarly, additional studies (66, 79, 93) have examined flow for the endocardium, midmyocardium, and epicardium after LCx ameroid placement and found no significant difference between resting flow of control and LCx regions up to 16 wk after ameroid placement. In the first of these studies (66), the authors did find a significant reduction in blood flow to all three myocardial layers within the LCx compared with control regions during exercise induced stress. In this same study, the endocardial layer only was underperfused during adenosine-induced coronary vasodilation. These reductions were stable up to 16 wk after ameroid constrictor placement (79, 93). Likewise, Görg and colleagues (29) found collateral flow during coronary vasodilation with adenosine to be ~20% of normal maximal flow at 4 wk postameroid placement, a value that increased to ~50–60% by 8 wk and did not increase further with longer time intervals up to 26 wk. Numerous additional studies that used radioactive microspheres to measure regional MBF within the ameroid occluded LCx distribution have confirmed regional perfusion to be normal at rest by ~3–4 wk postocclusion but MBF during stress to be consistently diminished compared with control regions of the heart (67, 78, 79, 85, 93).

Not surprisingly, given the lack of a significant reduction in myocardial blood flow at rest, regional function at rest, as assessed by using sonomicrometry to measure regional wall thickening, is similar to that of

![Fig. 3. Time course of changes in systolic wall thickening (WT) distal to a left circumflex (LCx) ameroid constrictor in conscious pigs during the development of progressive coronary artery stenosis.](http://jap.physiology.org/DownloadedFromRef.79) Reproduced from Ref. 79 with permission.
control regions at 3–16 wk after ameroid placement (66). In an elegant study, Shen and Vatner (79) measured regional myocardial function daily by using ultrasonic crystals after LCx ameroid placement in swine and found the peak reduction in systolic wall thickening (56 ± 6% of baseline) to occur at 20 ± 3 days after ameroid placement, after which it recovered toward normal. By 34 ± 2 days, regional systolic thickening was no different from baseline (Fig. 3). These authors also pointed out the variability in time to ameroid closure between animals as evidenced by differences in the time to peak reduction in systolic function and the importance of normalizing regional functional data to control regions within the same heart (79). Again similar to the blood flow data, numerous studies have shown regional function during stress in the collateral-dependent LCx region to be markedly reduced compared with baseline as well as control nonischemic regions (66, 78, 79). These changes are likewise stable up to at least 16 wk postoperatively (66).

White and colleagues (93) have examined in detail the morphometry and function of the collateral circulation developing after LCx ameroid constrictor placement in swine. By 3 wk after ameroid placement, there was a significant increase in the number of collateral vessels that originated from branches of the other two major coronary arteries (intercoronary) and from vessels outside the heart (extracardiac) such as the bronchial, internal mammary, and retrocardiac arteries. The majority of intercoronary collaterals were 20–60 μm in diameter with a medial thickness 50–70% of normal arterioles (due to significantly less smooth muscle in their walls), whereas the extracardiac collaterals tended to be somewhat larger and thicker walled (medial thickness 80% control). The number and size of the collateral vessels increased significantly from 3 to 8 wk after surgery but then remained stable to 16 wk. The intercoronary collaterals were uniformly distributed in the endocardium and midmyocardium with a clustering around the posterior papillary muscle, whereas the extracardiac collateral vessels were predominantly located in the epicardial region. The extracardiac collaterals were less numerous than their intercoronary counterparts. These collateral vessels resulted in a 14-fold increase in collateral-dependent flow over preexisting values before ameroid-induced coronary occlusion. The extracardiac collateral sources supply up to 30% of this value. DNA labeling studies of endothelial and smooth muscle cells demonstrated a 50- to 70-fold increase in the labeling index of both endothelial and smooth muscle cells 2–3 wk after ameroid placement, consistent with collateral development with a decrease in the labeling index back to baseline values by 8 wk. Unlike dog collaterals, which develop as “mature” collaterals with normal amounts of medial smooth muscle and which are able to restore normal blood flow to the ameroid-occluded distribution even under conditions of stress (31), the porcine collaterals with their relative lack of smooth muscle do not respond predictably to vasodilator therapy or stress. Consequently, the collateral reserve is limited, thus explaining the inducibility of ischemia with stress in the ameroid model. Prior work has demonstrated that these collaterals develop similarly in animals ranging from several months to several years old (20).

The major advantage of the ameroid constrictor model is its simplicity. Inherent limitations to the use of these occluders include an inability to control the rate or degree (sometimes incomplete) of coronary occlusion (45). This likely contributes to the large variability in the percent infarction of the area at risk as well as a mortality rate approaching 30% in those animals with more rapid coronary occlusion. In addition, as a model of human coronary artery disease, the ameroid model is limited as well. Despite a limited innate collateral circulation, collateral vessels in the pig appear to develop rapidly (Fig. 4) and restore myocardial blood flow and function at rest to normal levels by 3–7 wk after ameroid-induced coronary occlusion (60, 66). Although these collaterals are capable of supplying normal blood flow at rest, they are incapable of supplying adequate flow during periods of augmented myocardial oxygen demand and thus impaired left ventricular regional function results. Because the collateral vessels developed in the ameroid model in swine
provide insufficient blood flow during exercise, i.e., coronary flow reserve is impaired, the model is essentially one of stress-induced ischemic dysfunction (21, 66), similar to the clinical syndrome of effort ischemia. The normal flow and function at rest may limit the utility of this model for studies of proangiogenic therapies, and any studies must include an assessment of myocardial blood flow and function under conditions of stress pre- and posttreatment.

**Fixed stenosis model.** Unlike the ameroid constrictor, the fixed stenosis model has not been used for the assessment of proangiogenic therapies to date but rather has been widely utilized in studies investigating the pathophysiology of hibernating myocardium (14, 25, 26, 48). One rationale for using a fixed stenosis rather than a total occlusion model is that with a fixed degree of high-grade narrowing of a coronary artery in swine, unlike the situation with the ameroid constrictor, MBF in the region subtended by the stenotic artery is significantly decreased at rest (14, 26, 48). The reason for this discrepancy appears to be that fewer collaterals are formed with lesser degrees of occlusion (57). This is supported by prior experimental studies (7, 26) suggesting that significant collateral recruitment in swine does not occur in the absence of total coronary occlusion. This is similar to the situation in humans, in whom angiographically evident collaterals generally do not become apparent until epicardial coronary occlusion exceeds 90%, although heterogeneity clearly exists (51).

The methods for producing the fixed degree of stenosis have generally utilized some means of banding the coronary artery of interest to a fixed dimension. Chen and colleagues (13) used a silk tie placed around the proximal left anterior descending (LAD) coronary artery to reduce the outer diameter of the artery such that resting flow through the stenotic region was decreased by ~40%. Reductions in resting flow were stable for up to 24 h in this study and were accompanied by a significant reduction in wall thickening in the LAD distribution. Histology revealed minimal (~6% of area at risk) to no myocardial infarction with ultrastructural changes including partial loss of myofibrils and an increase in mitochondria and glycogen (Fig. 1) consistent with myocardial hibernation. After release of the stenosis at 24 h, wall motion recovered over the course of a week and the ultrastructural changes reverted to normal, both of which are consistent with the area at risk being ischemic yet viable. In a subsequent study (14), the same group demonstrated that these reductions in flow and regional wall thickening using this fixed LAD stenosis model were stable for up to 4 wk postoperatively. As in the previous study, there was little (~6%) to no infarct of the area at risk at 4 wk. Another interesting finding of this study was that ongoing myocyte apoptosis (programmed cell death) occurs in the distribution of the stenotic coronary artery, predominantly in the subendocardial myocardium. These findings suggest that ongoing cell death occurs in chronically ischemic yet viable myocardium and that apoptosis may in part be responsible for myocyte loss and increased fibrosis long-term in chronically hibernating regions. Consideration should be given to this process of apoptosis when planning studies investigating means of improving myocardial perfusion via proangiogenic therapies because studies with a prolonged period from the creation of coronary stenosis to treatment may be doomed to less vigorous improvements in regional function despite increased blood flow due to cardiomyocyte loss from programmed cell death. This latter point is also emphasized in a study by Lai and colleagues (48) using this same LAD stenosis model. These investigators found evidence for progressive left ventricular remodeling with increases in ventricular volume, mass, and interstitial fibrosis over a 4-wk period after stenosis creation. These changes were partially reversible after 3 wk of reperfusion. These authors postulated a mechanism whereby regional wall thinning and left ventricular cavity dilatation lead to increased wall stress and, through neural or endocrine activation, produce progressive myocyte degeneration and fibrosis if revascularization is not performed in a timely fashion.

Fallavollita et al. (26) have utilized a similar coronary stenosis model in which an occluder of fixed internal dimension (1.5–2.25 mm internal diameter of occluder) is placed about the proximal LAD. In this study, juvenile swine (average weight 8 kg) were instrumented with an LAD stenosis and then studied at 3 mo; at the time of death the mean weight of the animals was 75 kg. There was a high rate of total coronary occlusion by coronary angiography at 3 mo, which is not surprising given that the fixed occluders were placed on the LAD before significant growth of the animal (and vessel). Consequently, the relative degree of stenosis could be expected to progress as vessel diameter increased with increases in animal size. At 3 mo after placement of the occluder, regional wall motion was significantly depressed with severe hypo- to akinesis on left ventriculography. Microsphere analysis demonstrated significant reductions in regional myocardial blood flow (24% reduction in subendocardial and 11% reduction in transmural MBF). However, histology did not demonstrate light microscopic evidence for significant myocardial necrosis (~6% of area at risk), and the dysfunctional regions demonstrated recruitable inotropic reserve consistent with preserved viability. Likewise, regional fluorodeoxyglucose (FDG) deposition by PET was increased in the LAD distribution, consistent with preserved viability and ischemia (1), with a transmural variation in FDG uptake that was most pronounced in the subendocardium. Angiographic collaterals were noted to fill the proximally occluded LAD; sources of the collaterals including bridging vessels across the stenosis, LCx, and right coronary arteries. No angiographic collaterals were visible in the absence of total occlusion of the LAD. In a follow-up study, Fallavollita and Canty (25) studied animals at 1 or 2 mo after LAD banding using this same model. The degree of stenosis averaged 74 ± 5% at 1 mo and 83 ± 6% at 2 mo. When colored microspheres were used to measure MBF, resting per-
fusion was normal in the LAD distribution at both 1 and 2 mo. However, resting wall motion in these same regions was significantly reduced, consistent with chronic stunning. Vasodilator reserve was mildly reduced at 1 mo and markedly impaired by 2 mo after occluder placement. There was no difference in FDG uptake in the LAD distribution at 1 mo but a significant increase at 2 mo, consistent with ischemia and viability as noted above. Thus, on the basis of the results of these two studies, it appears that both the degree of stenosis and possibly the length of time the stenosis is present determine whether MBF in the distribution of the stenotic coronary artery is reduced or normal at rest, factors that must be taken into consideration in planning studies to test the ability of a proangiogenic therapy to augment regional perfusion.

The main advantage of the fixed stenosis model is that a relatively uniform degree of ischemia may be induced in all animals, which differs somewhat from the interanimal variability seen with the ameroid constrictor. A second advantage is that regional myocardial blood flow and function in the distribution of the stenotic coronary artery are reduced at rest, thus giving an additional parameter to assess for improvement after proangiogenic therapy. The major disadvantage is the lack of widespread use of the model to date with investigator unfamiliarity in the technically more demanding techniques necessary for producing a chronic, stable coronary stenosis.

**Hydraulic occluder model.** Another large-animal model of chronic ischemia has involved placing an adjustable hydraulic occluder around an epicardial coronary artery to produce a fixed degree of coronary stenosis (9). This model is similar to the fixed stenosis model in that the coronary artery is reduced in diameter by an external device, although with this latter model the occluder is typically placed either proximal or distal to a myocardial flow probe. The proximal end of the occluder is externalized at the time of surgery and then may be inflated with either air or liquid material once the animal is recovered from surgery. In this manner, the degree of coronary stenosis can be finely adjusted to reduce baseline flow through the epicardial coronary artery as measured by the flow probe by a given amount and thus produce a stenosis of the severity desired. Bolukoglu and colleagues (9) were among the first to employ this type of model in an attempt to reproduce the clinical condition of chronic hibernating myocardium. These investigators placed a Doppler flow probe and hydraulic occluder in sequence around the proximal LAD of swine. The occluder was then inflated during the surgical procedure to reduce flow velocity as measured by the flow probe located just proximal to the occluder by 50%. This stenosis was then maintained for 7 days. Perioperative mortality rate in this study was nearly 44% with deaths due to cardiac arrhythmias from coronary spasm after inflation of the occluder. For those animals surviving the 7-day period, systolic shortening in the area at risk was decreased by ~40%. Metabolic studies at 7 days indicated no evidence for acidosis or cell death, preserved contractile reserve during low-dose dobutamine infusion, and no infarction by histological exam, all of which suggest that the stenosis produced chronically ischemic yet viable myocardium in the LAD distribution.

Interestingly, cross sections of the LAD at the point of placement of the occluder demonstrated adventitial and medial injury with subendothelial fibroblast proliferation and elevation of overlying endothelial cells, consistent with occluder-induced vessel injury. These structural alterations within the vessel appeared to be functionally significant and potentially permanent, as there was no change in the velocity of blood flow through the region in which the occluder had been placed even after the stenosis was released at 7 days postoperatively. It is likely that these ultrastructural alterations in vessel architecture are common to both the ameroid constrictor and fixed stenosis models as well.

A modification of the model of Bolukoglu and colleagues (9) places a hydraulic occluder on the proximal LCx with an ultrasonic flow probe immediately distal to the occluder to measure downstream MBF (Fig. 5; Ref. 82). The flow probe uses transit-time ultrasound to continuously measure the actual volume rate of flow (11, 16) and allows precise adjustment of the degree of stenosis via the externalized portion of the hydraulic occluder. The ultrasonic probes are highly accurate, are atraumatic to the coronary artery, and overcome the limitations of electromagnetic and Doppler flow probes used in the past (16). Unlike the study by Bolukoglu et al., in this modified model (82) used extensively in our laboratory the animals are allowed to

![Fig. 5. Coronary angiogram demonstrating experimental preparation. Note location of hydraulic occluder (radiolucent and not visualized) producing a high-grade stenosis of the proximal LCx coronary artery. Normal-diameter LAD coronary artery is labeled for comparison. The ultrasonic flow probe is located downstream from the occluder on the LCx and allows the degree of stenosis to be adjusted to produce the desired reduction in epicardial flow. Reproduced from Ref. 82 with permission.](https://www.jap.org)
recover from surgery for several days before inflation of the hydraulic occluder, such that the stress response associated with surgery has had time to abate. This difference in experimental protocol may account for the high rate of fatal arrhythmias in the study by Bolukoglu et al., whereas in studies of hundreds of animals to date we have seen fatal arrhythmias after stenosis creation well less than 1% of the time (unpublished data).

This modified model has been well characterized and has been used to investigate the pathophysiological mechanisms of hibernating myocardium (43, 82), the efficacy of proangiogenic therapy with recombinant growth factors (8, 40), and the effects of laser (41, 42, 44) and mechanical (39) transmyocardial revascularization. A high-grade proximal LCx stenosis is produced by inflating the occluder such that flow through the artery as measured by the ultrasonic flow probe immediately distal to the occluder is reduced by ~90%. This degree of stenosis is then maintained chronically and results in an ~25–30% decrease in transmural MBF in the LCx distribution myocardium as measured by using quantitative PET with $^{13}$NH$_3$ (Fig. 6). These reductions in MBF have been maintained for up to 6 mo with no significant change in flow over this time period (41, 42). $^{[18F]}$FDG measurement of glucose utilization by PET consistently demonstrates a 120–140% increase in FDG uptake in the LCx myocardium consistent with ischemia as well as preserved viability (1, 10, 18) (Fig. 6).

Analysis of regional wall motion by transthoracic echocardiography of the LCx distribution typically reveals severe hypokinesis at rest. The dysfunctional segments show a significant improvement over rest function during low-dose dobutamine infusion (contractile reserve) followed by deterioration with high-dose dobutamine. This biphasic response of initial improvement followed by deterioration is characteristic of ischemic, viable myocardium (2, 15) (Fig. 7). Triphenyl tetrazolium chloride staining as well as light and electron microscopic techniques demonstrate little to no (0–8%) subendocardial infarct within the area at risk (82). Furthermore, electron microscopy reveals the previously described ultrastructural changes characteristic of chronically ischemic yet viable myocardium (37, 82, 88), including a loss of contractile material within cardiomyocytes with the space previously occupied by the myofilaments filled with glycogen, small mitochondria scattered throughout the myolytic cytoplasm, and tortuous nuclei with uniformly dispersed heterochromatin, among others. These changes are more prominent in the subendocardial regions (Fig. 1).

The major advantage of the hydraulic occluder model is the consistent degree of ischemia within the area at risk, likely secondary to the uniform coronary stenosis produced by using ultrasonic flow probe guidance. This consistency is aided by the fact that the stenosis is produced several days postoperatively once the animal has recovered from surgery and any coronary spasm associated with operative manipulation has subsided and thus gives a more accurate idea of the degree of stenosis produced. Also, the degree of coronary narrowing can be manipulated as needed, unlike the fixed stenosis model, in which postoperative adjustment is not possible. As with the fixed stenosis model, this model also produces a situation in which myocardial perfusion in the distribution of the stenotic coronary artery is generally reduced at rest, an advantage for studies investigating means to improve MBF. Additionally, this experimental preparation is associated with a low incidence of animal loss from cardiac death. The major disadvantage is that the model is technically demanding from both a surgical standpoint of dissecting out the coronary artery for occluder and flow probe placement as well as the standpoint of chronic animal maintenance given the externalized hardware.

ADDITIONAL CONSIDERATIONS

Many experimental protocols no longer use farm-bred swine because of numerous problems inherent to these animals, including a high susceptibility to malignant hyperthermia (58) and ventricular fibrillation.
after periods of excitement, rapid growth (94), and difficulty adapting to laboratory conditions (94). Because of these problems with the use of farm-bred swine, especially in chronic experiments, several species of miniswine have been developed that overcome many of these limitations (62, 83). We have found miniswine to be well suited to long-term survival studies and recommend them over farm-bred swine for any type of chronic study, including translational studies investigating the efficacy of proangiogenic techniques.

A second consideration regarding study design for assessing proangiogenic therapies regards the duration of ischemia before application of the angiogenic therapy. As noted above, chronically ischemic yet viable myocardium is characterized by progressive myocyte apoptosis (14), ventricular remodeling (48), and structural alterations including loss of contractile material within cardiomyocytes and increases in the amount of interstitial connective tissue (88). Presumably, these alterations are related to the diminished ventricular function observed in the ischemic regions, as studies have demonstrated that patients with more advanced cardiomyocyte deterioration have less return of function after revascularization than those with less advanced changes (22, 23, 32, 76). For example, Beanlands and colleagues (6) studied the effects of the timing of surgical revascularization on the recovery of left ventricular function and survival in patients with hibernating myocardium and found that delayed revascularization was associated with increased preoperative mortality and a lack of improvement in left ventricular function compared with those operated on early. Consequently, because of the progressive nature of these phenotypic alterations in the absence of revascularization, the period of experimentally induced ischemia in studies investigating proangiogenic therapies should probably not be excessively prolonged; otherwise, functional recovery may be limited despite improvement in regional perfusion. This would hamper efficacy studies for angiogenic agents.

Unger (87) and Post and colleagues (65) have recently reviewed the various potential experimental endpoints in studies of proangiogenic therapies. At a minimum, studies should include an assessment of regional myocardial perfusion and function pre- and posttreatment as well as histological evaluation of angiogenesis and arteriogenesis. Histological analysis should include immunohistochemical staining for endothelial cell-specific markers to confirm the presence of endothelial cells within suspected blood vessels (44).

Regarding serial measurements of myocardial perfusion, numerous studies in both animals and humans (12, 53) have demonstrated the spatial heterogeneity of absolute values of myocardial blood flow between different regions of the left ventricle as well as the inherent variability in serial measurements of absolute flow for a given region of the heart over time (17, 56). Consequently, in assessing potential changes in regional myocardial perfusion, normalization of the data, in which perfusion in the ischemic region of interest is expressed as a percentage of that in the nonischemic areas of the same heart, is preferred for ease of comparison of serial measurements given the temporal and spatial heterogeneity in absolute flow values (42, 67).

A wide variety of angiogenic therapies have been demonstrated effective in various porcine chronic ischemia models, including recombinant fibroblast (8, 47, 68) and vascular endothelial growth factors (40,
69), gene therapy techniques (54, 70), autologous bone marrow (28), and transmyocardial laser revascularization (35, 39, 41, 42, 44, 70), among others. These numerous experimental successes have been seen despite much less impressive results when using many of these same therapies in the clinical arena (33, 49, 50, 80). The reason for this discrepancy is unknown, but potential explanations may relate to differences between the animal models utilized and human subjects (81). Until recently, neovascularization in the chronically ischemic adult heart had been thought to be due to the processes of angiogenesis and arteriogenesis. Angiogenesis refers to the sprouting of new capillaries from preexisting ones and is mainly caused by hypoxia and mediated via activation of hypoxia-inducible factor, which serves to increase transcription of VEGF and its receptors and stabilize VEGF mRNA (72). Arteriogenesis, on the other hand, is the growth of arteries from preexisting arterioles; it is the type of vascular growth responsible for maturation of collateral conduits and produces vessels capable of carrying significant blood flow as well as being visualized with angiography (27, 72). Primary arteriogenic stimuli include shear stress and inflammation in which an invasion of monocytes and other white blood cells leads to the production of growth factors such as the fibroblast growth factors (FGF) with subsequent vascular growth (71, 73). Recent work (4, 5), however, has demonstrated that neovascularization in adults is not restricted to angiogenesis and arteriogenesis but rather involves vasculogenesis as well (27, 63). Vasculogenesis refers to the process of in situ formation of blood vessels from endothelial progenitor cells termed angioblasts (27, 63). Angioblasts migrate and fuse with other endothelial progenitor cells and capillaries to form a primitive network of vessels known as the primary capillary plexus. After this primary capillary plexus is formed, it is remodeled by sprouting and branching via the process of angiogenesis. Thus angiogenesis, vasculogenesis, and arteriogenesis all potentially contribute to neovascularization in the adult heart (27), although some authors (71) have suggested that arteriogenesis is necessary for significant improvements in myocardial blood flow and ultimately represents the desired effect of “therapeutic coronary angiogenesis.” However, most studies on arteriogenesis have focused on areas in which preexisting collaterals are prevalent such as in the peripheral extremities, and it remains to be seen whether the same conditions are required in the heart or whether de novo formation of collaterals is the predominant mechanism. Regardless, because by definition arteriogenesis requires a source arteriole from which to grow, human subjects with so-called “end-stage” coronary artery disease may lack the appropriate substrate (81). In addition, the processes of angiogenesis and vasculogenesis may be impaired in these individuals as well (27). As discussed in this review, the experimental models typically utilized in translational studies of proangiogenic agents involve young, healthy animals with single-vesSEL coronary disease in which the remaining vessels are normal and thus potentially more able to sprout collateral vessels capable of improving myocardial blood flow to the ischemic regions. These differences may explain the disparate results seen to date in animal vs. human studies. In addition, they suggest that animal models of multivesSEL CAD may need to be developed to provide a more rigorous assessment of therapies appearing promising in single-vascular disease models.

Finally, neovascularization represents a highly ordered physiological mechanism under tight regulation with many factors active at the molecular level to influence the process (63), including numerous soluble polypeptides such as VEGF, angiopoietins, FGF, platelet-derived growth factors, transforming growth factor-β, tumor necrosis factor-α, and colony-stimulating factors, as well as many others. In addition, several membrane-bound proteins play prominent roles in angiogenesis, including various members of the integrin, cadherin, and ephrin families. Finally, mechanical forces acting on the endothelium also contribute to the regulation of angiogenesis (63). The importance of further investigation into the interactions of these regulatory processes and their potential modification for therapeutic benefit cannot be overemphasized. In addition, genomic and proteomic approaches will take on an ever-important position in the field of angiogenesis research in the future (30, 64). Consequently, the physiological and anatomic benefits of porcine models need to be weighted against smaller animal models such as rats and mice in which there is a far greater ability to perform studies in defined genetic models or apply genomic and/or proteomic techniques.

In summary, the field of therapeutic angiogenesis remains in its infancy, with moderate successes being reported in the clinical arena in some instances and disappointments in others. If the field is to advance, scientifically rigorous translational studies carried out in appropriate animal models will be necessary to develop and assess potential proangiogenic therapies before their use in humans. Although continued work using small animal models such as mice remains important, the ultimate test of therapeutic efficacy in the preclinical setting involves large-animal models of myocardial ischemia. Consequently, we hope the information contained in this review will help investigators make informed decisions regarding the planning of future studies such that these therapies may one day find their way into everyday clinical practice.

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