TRANSLATIONAL PHYSIOLOGY

The bottleneck stent model for chronic myocardial ischemia and heart failure in pigs

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Rissanen TT, Nurro J, Halonen PJ, Tarkia M, Saraste A, Rannankari M, Honkonen K, Pietilä M, Leppänen O, Kuivanen A, Knutti J, Ylä-Herttuala S. The bottleneck stent model for chronic myocardial ischemia and heart failure in pigs. Am J Physiol Heart Circ Physiol 305: H1297–H1308, 2013. First published August 30, 2013; doi:10.1152/ajpheart.00561.2013.—A large animal model of chronic myocardial ischemia and heart failure is crucial for the development of novel therapeutic approaches. In this study we developed a novel percutaneous one- and two-vessel model for chronic myocardial ischemia using a stent coated with a polytetrafluoroethylene tube formed in a bottleneck shape. The bottleneck stent was implanted in the proximal left anterior descending (LAD) or proximal circumflex artery (LCX), or in both proximal LCX and mid LAD 1 wk later (2-vessel model), and pigs were followed for 4–5 wk. Ejection fraction (EF), infarct size, collateral growth, and myocardial perfusion were assessed. Pigs were given antiarrhythmic medication to prevent sudden death. The occlusion time of the bottleneck stent and the timing of myocardial infarction could be modulated by the duration of antiplatelet medication. Fractional flow reserve measurements and positron emission tomography imaging showed severe ischemia after bottleneck stenting covering over 50% of the left ventricle in the proximal LAD model. Complete coronary occlusion was necessary for significant collateral growth, which mostly had occurred already during the first wk after the stent occlusion. Dynamic and competitive collateral growth patterns were observed. EF declined from 64 to 41% in the LCX model and to 44% in the LAD model 4 wk after stenting with 12 and 21% infarcted left ventricle in the LCX and LAD models, respectively. The mortality was 32 and 37% in the LCX and LAD models but very (71%) high in the two-vessel disease model. The implantation of a novel bottleneck stent in the proximal LAD or LCX is a novel porcine model of reversible myocardial ischemia (open stent) and ischemic heart failure (occluded stent) and is feasible for the development of novel therapeutic approaches.

angiogenesis; arteriogenesis; collateral growth; ischemia; myocardium

A CLINICALLY RELEVANT and reproducible large animal model is crucial for the development of novel therapies for chronic myocardial ischemia and ischemic heart failure. The surgical implantation of an ameroid constrictor around the proximal left anterior descending (LAD) or proximal circumflex artery (LCX) to cause gradual coronary occlusion, myocardial ischemia, and infarction has been used already for decades (10). However, this model requires thoracotomy, making it laborious and painful for animals, and cardiac surgery causes the risk of severe infection and adhesive pericarditis, limiting the possible interventions. The most important drawback of this model is that the coronary occlusion rate is highly variable, and only 36% of the coronary arteries are occluded 1 mo after the placement of the ameroid (16). Because of this variation, large numbers of animals are required for sufficient statistical power. In a variation of this model, the ligation of the distal LAD before the ameroid implantation around the proximal LAD led to more reducible results (22).

Because of the limitations in the previous models, we sought to develop a novel large animal model for chronic myocardial ischemia, which would be consistent, cause ischemic heart failure, induce collateral growth, be less painful, and be percutaneous, allowing an easy and rapid procedure. Similarly, with the surgical ameroid model, the objective of a catheter-mediated model is to achieve a gradual total occlusion in the proximal coronary artery that causes myocardial ischemia and infarction and also launches collateral artery growth.

MATERIALS AND METHODS

Medication. All animal experiments were approved by the National Animal Experiment Board in Finland and conform to the Directive 2010/63/EU of the European Parliament. Domestic pigs weighing 30–35 kg were used (n = 50). The study groups are shown in Table 1. Pigs were sedated with atropine (0.05 mg/kg im; Leiras, Helsinki, Finland) and azaperone (Stresnil, 8 mg/kg im; Janssen, Titusville, NJ). Pigs were then anesthetized with propofol (Propofol-Lipuro, 15 mg·kg⁻¹·h⁻¹ iv; B. Braun Medical, Melsungen, Germany), intubated, and mechanically ventilated. Fentanyl (10 μg·kg⁻¹·h⁻¹ iv; Janssen), buprenorphine (Temgesic, 0.3 mg im; Janssen), and midazolam (Versed, 0.05 mg/kg im; Hospira, Lake Forest, IL) were used for analgesia. The level of anesthesia and analgesia was monitored by experienced animal technicians.

Administration of amiodarone (Cordarone, 200 mg/day po; Sanofi-Aventis, Paris, France) and bisoprolol (Bisoprolol-ratiopharm, 2.5 mg/day po; Ratiopharm, Ulm, Germany) was started 1 wk before...
The arterial sheath was removed after the pig had been transferred caudal 30° (CAUD30) and let anterior oblique 40° (LAO40); views used for the RCA were left anterior oblique 10° (LAO10) and left coronary arteries (RCA and LCA, respectively) were cannulated (3-D) angiography device (GE Healthcare, Waukesha, WI). Because of MgSO4 (Addex-magnesium sulfate, 246 mg/ml; Fresenius Kabi, Uppsala, Sweden) immediately before stenting to prevent arrhythmias. All pigs received acetylsalicylic acid (ASA-riatropham, 300 mg po; Ratiopharm) and clopidogrel (Clopidogrel Mylan, 300 mg po; Mylan, Saint Priest, France) 1 day before the procedure. Enoxaparin (Klexane, 30 mg iv, Sanofi-Aventis) was administered after the insertion of an introducer sheath in the right femoral artery, and another 30 mg were given subcutaneously after the sheath was removed and hemostasis was secured.

In the LCX-stented pigs receiving dual-antiplatelet regimen (DAPT) throughout the experiment (DAPT+ group), ASA (100 mg/day po), clopidogrel (75 mg/day po), and enoxaparin (30 mg/day sc) were continued for the entire duration of the experiment. In the DAPT− group, only the loading doses of ASA and clopidogrel were given (Table 1). In the LAD-stented pigs that were used for positron emission tomography (PET) imaging, DAPT was given for 1 wk after stenting to keep the stent patent until the first PET imaging and was discontinued thereafter. For the rest of the LAD-stented pigs used for other analyses, only loading doses of DAPT were given. The prophylactic antibiotic cefuroxime (Zinacef, 500 mg im; GlaxoSmithKline, Brentford, UK) was given before every invasive procedure. Sublingual dinitrate (Dinit, 1.25 mg/dose; Leiras) was given before catheterization.

**Catheterization of the pigs.** Surgical dissection of the carotid artery has often been used for arterial access in pigs (4, 18, 24, 26). However, we used the normal percutaneous femoral approach because it is faster, causes less radiation to the operator, is less painful for the animals, and allows follow-up angiographies (2, 9, 13). Femoral introducer sheaths (12 cm in length; Cordis, Bridgewater, NJ) were placed percutaneously in the right femoral artery (6-F) and vein (4-F) using the standard Seldinger’s technique.

Catheterization of the pigs was done in a laboratory dedicated for animal use and equipped with a GE Innova 3100™ three-dimensional (3-D) angiography device (GE Healthcare, Waukesha, WI). Because of the narrow aortic arch in pigs, suitable catheters for coronary angiography are short, curved Amplatz-type catheters (13). The right and left coronary arteries (RCA and LCA, respectively) were cannulated using 5-F AR-1 diagnostic coronary catheter (Medtronic, Albany, NY) and 6-F AR-2 guiding catheter (Laucher; Medtronic). The views used for the RCA were left anterior oblique 10° (LAO10) caudal 30° (CAUD30) and let anterior oblique 40° (LAO40); views for the LCA were right anterior oblique 90° (RAO90) and LAO40. The arterial sheath was removed after the pig had been transferred from the angiography table to the floor for awakening. The Femostop device (St. Jude Medical, St. Paul, MN) was used to secure hemostasis according to the manufacturer’s instructions. Attention was paid to careful hemostasis because bleeding complications are often fatal in pigs with the use of DAPT (9, 13).

The Rentrop score of collateral growth was calculated as previously described (17). For left ventricular cineangiography (LV-cine), 30 ml of contrast agent at the rate of 10 ml/s (Hexabrix, 320 mg I/ml; Guerbet, Aulnay-sous-Bois, France) was injected via a 5-F pigtail catheter positioned in the LV. LV-cine was performed at rest and during dobutamine stress after the heart rate of 160/min was achieved with dobutamine infusion (10–40 μg·kg⁻¹·min⁻¹, Dobutamin Hameln 12.5 mg/ml; Hameln Pharmaceuticals, Hameln, Germany). The LV ejection fraction (LVEF) and the end-diastolic volume of the LV (LVEDV) were calculated by the analysis software of the GE Innova angiography device. Pigs were euthanized under deep anesthesia with 60 ml of intravenously administered saturated KCl solution. For postmortem 3-D angiogram, both coronary arteries were catheterized via contralateral femoral arteries in a subset of animals. Immediately after asystole was achieved, bilateral contrast injection was performed and rotational 3-D angiography was acquired.

**Bottleneck stent model for myocardial ischemia.** The bottleneck stent consists of a 3–4 × 8-mm bare metal stent (BMS) covered by polytetrafluoroethylene (PTFE) heat shrink tubing (diameter 5/64 in.; Fluoroplast, Petalax, Finland), with the other end shaped into a bottleneck form to restrict coronary blood flow (Fig. 1). Similar heat shrink tubing can be obtained via several providers in Europe and the U.S. To create the bottleneck shape, a standard 22-gauge (0.7 mm) or 20-gauge (0.9 mm) syringe needle was inserted inside the other end of the PTFE tube and the tube was heated to a temperature of 315°C, causing the tube to shrink around the needle, which was then removed. After cooling to room temperature, the narrow part of the bottleneck was cut to a length of 2 mm and the other end of the tube was cut so that when the bottleneck was placed over the stent, one stent strut would be left outside the tube.

To create a coronary stenosis of 0.7 or 0.9 mm in diameter, a sterilized PTFE tube with a corresponding bottleneck diameter was inserted on either a Coroflex Blue Ultra (B. Braun Medical; profile 0.8 mm) or Multilink Vision (Abbott Laboratories, Abbott Park, IL; profile 1.0 mm) bare metal stent. Because the profile of the nondilated stent is larger than the diameter of the bottleneck tube orifice, the tube does not slide over the stent balloon during the delivery of the construct. To create a coronary artery stenosis, the construct was delivered into the coronary artery via the 6-F AR-2 guiding catheter and a standard 0.014-in. guide wire. The construct was dilated at 12 ATM in the target coronary artery, and the stent balloon was withdrawn (Fig. 1; Supplemental Videos 1 and 9). (Supplemental data is available online at the American Journal of Physiology-Heart and Circulatory Physiology website.) The whole stenting procedure including the insertion of the introducer sheath and coronary angiography took ~10–15 min per animal.

For fractional flow reserve (FFR) measurements, the guide wire used was the Pressurewire Certus (St. Jude Medical). After administration of nitroglycerin (200 μg intracoronary), a 150-μg bolus of adenosine (Adenosin Life Medical; Life Medical Sweden; Stock- sund, Sweden) was given intracoronarily and the FFR value was recorded according to the manufacturer’s instructions (St. Jude Medical). FFR < 0.80 is generally used as a cutoff value to show myocardial ischemia and is an indication for revascularization (23).

**Positron emission tomography.** PET and computed tomography (CT) studies were performed with Discovery VCT hybrid PET/CT scanner (GE Medical Systems, Milwaukee, WI). Myocardial perfusion was evaluated by PET with 15O-radiolabeled water (957 ± 78 MBq) both at rest and during adenosine stress (200 μg·kg⁻¹·min⁻¹ iv infusion) early (2–4 days) and late (5 wk) after stenting as previously described (21). Myocardial viability was assessed by 18F-labeled fluorodeoxyglucose (18F-FDG) PET 5 wk

### Table 1. Number of pigs in each group and duration of DAPT

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Duration of DAPT</th>
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<tbody>
<tr>
<td>Animals used for model development</td>
<td>12</td>
<td>4 wk</td>
</tr>
<tr>
<td>LCX DAPT+</td>
<td>8</td>
<td>4 wk</td>
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<tr>
<td>LCX DAPT−</td>
<td>7</td>
<td>Loading dose</td>
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<tr>
<td>LAD</td>
<td>8</td>
<td>Loading dose</td>
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<tr>
<td>LCX + mid LAD</td>
<td>7</td>
<td>Loading dose</td>
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<tr>
<td>LAD for PET</td>
<td>8</td>
<td>1 wk</td>
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Animals stented in the left anterior descending coronary artery (LAD) for positron emission tomography (PET) imaging received dual antiplatelet therapy (DAPT) for 1 wk after bottleneck stenting to demonstrate the patency of the stent and inducible ischemia. DAPT included 100 mg/day aspirin and 75 mg/day clopidogrel (+ enoxaparin 30 mg sc). The loading dose was given 1 day before the procedure (300 mg aspirin, 300 mg clopidogrel, and 30 mg enoxaparin iv + sc). LCX, left circumflex artery.
after the stenting. To standardize myocardial glucose utilization, 1 g/kg glucose and 10 IU of insulin (Actrapid, 100 units/ml; Novo Nordisk, Bagsvaerd, Denmark) were administered intravenously immediately before tracer injection. Images were acquired for 15 min starting 40 min after injection of 275 ± 11 MBq of 18F-FDG. Coronary anatomy was evaluated by CT angiography with iodinated contrast agent (Omnipaque, 350 mg I/ml; Amersham Health, Oslo, Norway).

Fig. 1. Bottleneck stent model for chronic myocardial ischemia and heart failure in pigs. A: the bottleneck stent is advanced via an AR-2 guide catheter and guiding wire in the target coronary artery. LAD, left anterior descending coronary artery; LCX, left circumflex coronary artery; RCA, right coronary artery. B: the stent is dilated in the proximal coronary artery. C: the wire and guide catheter are withdrawn, and blood flow in the target coronary is immediately reduced due to the bottleneck (arrows), causing reversible myocardial ischemia. D: after discontinuation of the dual-antiplatelet therapy (DAPT), the stent gets occluded by thrombus formation, causing myocardial infarction. Collaterals are formed first from the RCA and later from the ipsilateral coronary artery. E: dilated 3.0-mm bare metal stent (BMS) covered by a polytetrafluoroethylene (PTFE) tube with the other end shaped into a bottleneck form to restrict coronary blood flow. F: bottleneck stent implantation in the proximal LCX (arrow). G: a significant stenosis in the proximal LCX immediately after stenting (arrow). H: bottleneck stenting in the proximal LAD (arrowhead). I: significant stenosis in proximal LAD immediately after stenting (arrow). J: bottleneck stent implantation in the mid LAD (arrowhead) 1 wk after stenting of the LCX, which has already been occluded (arrows in F and G). K: stenosis of the mid LAD immediately after stenting (arrowhead). Views for F–I are right anterior oblique 90° (RAO90), and views for J and K are left anterior oblique 40° (LAO40).
The acquired PET data were reconstructed with an iterative VUE Point algorithm. There were 2 iterations and 28 subsets in the reconstruction. The whole transaxial field of view (70 cm) was reconstructed in a $128 \times 128$ matrix, yielding a pixel size of $5.47 \times 5.47$ mm. The measurements were corrected for scatter, random counts, and dead time. The device produces 47 axial planes with a slice thickness of 3.27 mm.

Regional myocardial perfusion (in ml·g$^{-1}$·min$^{-1}$) and $^{18}$F-FDG uptake were measured using Carimas 2.0 software (Turku PET Centre, Turku, Finland; http://www.turkupertcentre.fi/carimas) as previously described (11, 21). The difference in global resting myocardial perfusion between 1 and 5 wk may need to be taken into account when evaluating the potential effects of collateral formation on myocardial perfusion. Interpretation of absolute myocardial blood flow values is difficult at this time because of the small number of observations, variable hemodynamic conditions, and lack of established normal values in the pig.

Infarcted and ischemic myocardial areas were defined as regions with myocardial blood flow less than 70% of maximum at rest and stress, respectively. On the basis of FDG uptake, myocardium was graded as viable, partially viable, or nonviable (relative FDG uptake 85, 67–85, and 67%, respectively).

Tetrazolium chloride staining for myocardial infarction. For quantification of infarcted myocardium, tetrazolium staining was performed. After death, the heart was sliced horizontally to 5-mm-thick samples, which were then incubated for 20 min at 37°C in 1% triphenyltetrazolium (catalog no. T8877; Sigma) diluted in PBS (pH 7.4). The viable myocardium is stained red, whereas infarcted tissue has a pale color. Digital images of the stained myocardial slices were obtained, and the infarcted area of the LV was quantified (AnalySIS, Germany).

Statistical analysis. Results are means ± SD. Statistical significance was evaluated by one-way ANOVA followed by an independent samples $t$-test or the Kruskal-Wallis test, followed by the Mann-Whitney $U$-test where appropriate. Nonparametric Pearson correlation analysis was used. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Bottleneck stent for induction of myocardial ischemia and collateral growth in pigs. Figure 1E illustrates the dilated bottleneck stent. The stent construct is very simple and could be implanted in the proximal LCX (Fig. 1, F and G) or LAD (Fig. 1, H and I) using a normal 6-F guiding catheter and a percutaneous coronary intervention (PCI) wire, resulting in a hemodynamically significant coronary stenosis (Supplemental Videos 1, 2, 9, and 10). In the two-vessel model, the second bottleneck stent was implanted in the mid LAD 1 wk after stenting of the proximal LCX (Fig. 1, J and K). In the pilot animals, the FFR measurements showed that the stent with a smaller orifice (0.7 mm) caused a more significant myocardial ischemia ($\text{FFR} = 0.64 \pm 0.15$), whereas the stent with a larger orifice (0.9 mm) induced hemodynamically a milder stenosis with $\text{FFR} = 0.77 \pm 0.07$ (Fig. 2). Thus the 0.7-mm orifice was chosen for the rest of the work.

The patency of the stents was 73 and 50% at 1 and 4 wk in the DAPT+ group, respectively, whereas all stents were already occluded at 1 wk in the DAPT− group. In the autopsies of the pigs that died suddenly, it was found that the stent was acutely occluded by red and white thrombus formation. At death 4 wk after the operation, histology of the occluded arteries showed reactive intimal hyperplasia, inflammation, and organized thrombus (see Fig. 6, I and J). Surprisingly, robust collateral artery growth was found after bottleneck stenting (Fig. 3; Supplemental Videos 3–7). First, collaterals from the RCA were formed, whereas bridging collaterals and collaterals from the LAD were weak. However, by 4 wk, bridging LCX-LCX collaterals and collaterals from the LAD were strengthened, whereas those from the RCA had subsequently regressed (Fig. 3, A–G). Three-dimensional reconstructions of coronary arteries demonstrated that RCA collaterals grew in the endocardial surface of the heart before connecting back to the recipient epicardial LCX (Fig. 3, H and I; Supplemental Video 8).

In animals with a proximal LAD stenosis and later a complete occlusion, arteriogenesis was also surprisingly fast, and collaterals were formed from the septal branches of the LAD as well as from the LCX and RCA (Fig. 4; Supplemental Videos 11–15). Similarly to the LCX model, collaterals in the LAD model had been formed almost completely by 1 wk after stenting. Interestingly, control angiograms revealed that collateral artery growth was a dynamic phenomenon. In the case of strong antegrade bridging of collaterals to provide blood pressure distal to the occlusion, there was less or even no collateral growth from the contralateral coronary artery (Fig. 5, A and B). Also, when large transmural infarction occurred due to abrupt thrombosis of the bottleneck stent, collateral growth was scarce because scarred myocardium requires little blood flow (Fig. 5, C–F).

Occlusion of the bottleneck stent caused either subendocardial or transmural myocardial infarction as shown by LV-cine and tetrazolium staining (Fig. 6; Supplemental Videos 16–19). After proximal LCX stenting (DAPT+), the typical finding was posterolateral subendocardial infarct with little effect on LV function (Fig. 6, A and B; Supplemental Video 16). In the DAPT− group, a common result was a transmural posterolateral infarct, which also clearly decreased LV function (Fig. 6, C and D; Supplemental Video 17). In pigs with the proximal LAD occlusion (discontinued DAPT), there was typically a substantial transmural anteroseptal infarct, leading to a severely depressed LV function (Fig. 6, E and F; Supplemental Video 18). Pigs receiving a bottleneck stent first in the proximal LCX and 1 wk later in the mid LAD had infarcts in both the anteroseptal and posterolateral regions (Fig. 6, G and H; Supplemental Video 19).

Effect of bottleneck stenting in LVEF, infarction area, survival, and collateral growth. The baseline LVEF in pigs was found to be 64% at rest and 78% during dobutamine stress with
LV-cine (Fig. 7A). LVEF did not significantly change in pigs after LCX stenting and continuous DAPT treatment (DAPT+) throughout the whole study (Fig. 7A). In contrast, LVEF was severely depressed after bottleneck stenting of the proximal LCX in the DAPT− group (LVEF 39 and 41% at 1 and 4 wk, respectively) and in the LAD (LVEF 41 and 43%, respectively). LVEF was mildly improved during dobutamine stress in these groups (Fig. 7A). In animals with two vessel occlusions (proximal LCX and mid LAD), LVEF was also significantly decreased (Fig. 7A).

The stenting of the proximal LCX (DAPT−) or the LAD as well as the combined LCX and mid-LAD stenting re-
sulted in 12, 21, and 14% infarct areas of the LV, respectively, as calculated by the tetrazolium staining (Fig. 7B). This was accompanied by a significant increase in LVEDV, suggesting the development of ischemic cardiomyopathy 4 wk after stenting. The infarct area was small (4%) in pigs in the DAPT/H11001 group due to increased patency of the bottle-neck stents. The mortality of pigs with only one coronary artery being stented was acceptable (32 and 37% in the LCX and LAD groups, respectively), with most of the deaths occurring during the first week after the procedure. In contrast, the pigs with two vessels stented tended to die (71%) after the second procedure, with a poor overall survival (Fig. 7C). All the deaths during the follow-up were sudden, probably due to fatal arrhythmias. Quantitative analysis of collateral growth using the Rentrop score showed that the maximal collateral growth was reached already during the first week after stenting, and no further increase was observed after 4 wk, except in the LCX DAPT+ group in which 50% of stents were still patent 1 wk after stenting, and therefore arteriogenesis was lesser at this time point (Fig. 7D). We found a trend in the correlation between the summed (RCA + LCA) Rentrop collateral score and ejection fraction ($P = 0.16$).

PET imaging of myocardial perfusion and viability after bottleneck stenting of the proximal LAD. The presence of regional myocardial perfusion abnormalities caused by bottleneck stent implantation in the proximal LAD was evaluated by PET perfusion imaging and coronary CT angiography (Fig. 8). At 1 wk, there were large perfusion defects (on average 53% of the LV) during adenosine stress in the anteroseptal region matching the LAD territory (Fig. 8, B, F, and H). Compared with stress, smaller defects (on average 24% of the LV) were seen at rest at 1 wk, indicating the presence of mostly reversible ischemia in the LAD region (Fig. 8, A and H). In these pigs, DAPT was continued for the first week after stenting and discontinued thereafter. At 5 wk after stenting (4 wk after discontinuation of DAPT), large anteroseptal defect was present at both stress and rest images (on average 54 and 42% of the LV, respectively), indicating the presence of large myocardial infarction with only some reversible ischemia in the border regions (Fig. 8, C–F and G). Quantitative analysis of the PET data showed that myocardial perfusion was higher during adenosine stress than at rest 1 wk after the procedure in the LCX and RCA regions ($P < 0.01$, Fig. 8H). At 5 wk, there was global reduction in myocardial perfusion compared with 1 wk ($P =
0.02 and 0.02 at rest and stress, respectively, Fig. 8J). Consistent with the perfusion data, $^{18}$F-FDG PET demonstrated that 64% of the LV was nonviable or only partially viable at 5 wk after bottleneck stenting in the proximal LAD (Fig. 8K).

**DISCUSSION**

Large animal models are necessary for the development of novel therapeutic approaches for the treatment of myocardial ischemia and heart failure, and the pig has been used most extensively. However, chronic myocardial ischemia and extensive infarction are difficult to achieve in pigs for several reasons. Most importantly, pigs have only a few endogenous collateral anastomoses, making them susceptible to transmural myocardial infarction (15, 19). Pigs are also remarkably vulnerable to sudden cardiac death caused by ventricular arrhythmias in case of a large ischemic area. The surgical ameroid constrictor model of myocardial ischemia, developed in 1957,
Fig. 6. Extent of myocardial infarction 4 wk after bottleneck stenting as shown by left ventricular cineangiography (LV-cine) and tetrazolium staining. A and B: bottleneck stenting of the LCX with efficient collateral formation typically leads to posterolateral subendocardial infarction (asterisk) without significant impairment of LV function (dashed area shows the difference between diastole and systole). See Fig. 2 for coronary angiography of the same animal. C and D: in the absence of collaterals, proximal LCX occlusion leads to transmural infarction with hypokinesia of the posterior wall (asterisks). See Fig. 4, C–F, for angiography of the same animal. E and F: large anteroseptal infarction and akinesia in the anterior wall in LV-cine after occlusion of the proximal LAD stent (asterisks). G and H: after stenting of both the LCX and mid LAD, transmural anteroseptal infarction (arrowhead) and posterolateral subendocardial infarction (asterisks) are visible. I and J: hematoxylin-eosin staining of the occluded LCX 4 wk after the operation at the location of the bottleneck (B), which was removed before tissue preparation. Arrows indicate bridging collaterals around the occluded LCX. Reactive intimal hyperplasia (asterisk), inflammation, and thrombus (arrowheads) are visible after stenting and occlusion of the vessel. Scale bar, 100 μm. Views are RAO90.
is the most widely used ischemia model, but it has several limitations (10). Other surgical models include, for example, a model in which the distal LAD is first ligated and an ameroid constrictor then placed in the proximal LAD (22). Although shown to lead to more reproducible results, this approach requires two surgical operations. As catheterization laboratories have recently become available for animal use, the interest in percutaneous approaches to induce myocardial ischemia has increased and led to the development of less invasive models.

The first percutaneous myocardial ischemia model was published in 1981 (8). In this study, a small 5-mm heparinized plastic truncated cone with a central internal lumen (diameter 0.625 mm) was passed over a guide wire into the LAD of six pigs. Diminished stress perfusion was demonstrated by the microsphere method, but the animals were followed only for 5 h before death. More recently, a PTFE stent graft with a ligature in the center was percutaneously implanted in the LAD, resulting in an hourglass-shaped stenosis of 75% (24). The total occlusion of the stent took place somewhere between 1 and 4 wk due to continuous DAPT and low-molecular-weight heparin medication throughout the study. The total infarction size was only 2% of the LV, and the mortality rate was 25%. The same “reduction stent” in the LCX resulted in 3% infarction area and 30% mortality rate (12). The degree of myocardial ischemia was not documented in these studies. In a similar approach a stent partly covered by an expanded PTFE membrane and a metal ring in the middle to prevent stent expansion was introduced in the LCX (4). The LCX was completely occluded 2 wk later with a stainless steel ball. The drawback of this model is that the total coronary occlusion required multiple instrumentations.

We have also tested the implantation of a reduction stent with a ligature in the middle of the stent. However, the major problem in this model is that the tight ligature needed for a hemodynamically significant stenosis makes the withdrawal of the stent balloon very difficult, if not impossible. Thus we figured out that we could circumvent this problem by using a construct in which the flow-limiting part is distal to the stent. The bottleneck form of the stent construct allows easy stent balloon withdrawal despite a very tight stenosis and induces remarkable reversible myocardial ischemia as shown by FFR measurement and PET imaging. The operation can be done via the femoral artery using normal PCI equipment and takes only 10–15 min per animal. A very important aspect of this model is the timing of the stent occlusion, because it affects the infarct size, ejection fraction, extent of collateral artery formation, and mortality. The occlusion of the stent can be modulated by altering the duration of DAPT; the occlusion rate was 100% already at 1

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**Fig. 7. Quantitative analysis of LV function, infarction area, survival, and collateral growth in the bottleneck stent model.**

A: bottleneck stenting of LCX with DAPT (DAPT+) throughout the study did not lead to significant reduction in LV ejection fraction (LVEF) as assessed by LV-cine at rest and during dobutamine stress. In contrast, placement of the bottleneck stent in the proximal LCX or in the LAD and discontinued antplatelet treatment at stenting (DAPT− group) led to a significant reduction of LVEF at rest. During dobutamine stress, LVEF was increased but stayed below the level of healthy animals. Sequential stenting of both LCX and mid LAD resulted in severe deterioration of LV function both at rest and during stress, implicating severe ischemic cardiomyopathy.

B: infarcted area was calculated in tetrazolium-stained slices of myocardium (see Fig. 5). LCX animals in DAPT+ group developed 3 times larger myocardial infarction than LCX animals of DAPT− group. The animals with proximal LAD stenting had the largest infracted area, covering 21% of the LV. The end-diastolic volumes of the LV increased accordingly in line with the extent myocardial injury. C: stenting of LCX and LAD caused a high mortality rate (71% at 4 wk) despite antiarrhythmic medication including continuous peroral β-blocker and amiodarone, whereas the mortality rate of the other groups was acceptable (32% in LCX and 37% in LAD model). D: collateral artery growth is surprisingly rapid in pigs. The majority of collaterals from the contralateral RCA or ipsilateral side (LCA, left coronary artery) have already been formed during the first week after bottleneck stenting of the LCX. *P < 0.05; **P < 0.01; ***P < 0.001.
wk with only a loading dose of DAPT but 50 and 73% at 1 and 4 wk, respectively, with continuous DAPT in the LCX.

A gradually evolving coronary stenosis has been generated by reactive intimal hyperplasia by polymer- or copper-coated stents (6, 9, 20, 26). Implantation of a copper-coated stent in the mid LCX caused chronic total occlusion within 4 wk with a low mortality rate of 6% and formation of bridging collaterals growing over the occlusion (20). In another study 68% of pigs developed a coronary stenosis of over 75% by 6 wk, but myocardial ischemia and the formation of collaterals were not reported (26). A custom-made stent composed of 0.3-mm-thick copper wire placed in the proximal LAD caused over 90% stenosis by ~3 wk in half of the pigs (9). LVEF was reduced from 68 to 25% 3 wk after the operation at the cost of a 36% mortality rate. The major difference between this model and the bottleneck model is the large variation in the time needed for the development of a hemodynamically significant stenosis in the copper wire model. In contrast, the implantation of the bottleneck stent induces myocardial ischemia immediately, and the stents were occluded at the end of the follow-up if DAPT was discontinued.

LV dysfunction has been generated via acute myocardial infarction by using an occlusion coil or gelatin sponge in the mid to distal LAD, which has been shown to decrease LVEF from 65 to 50% (13, 14, 18, 25). The more proximal acute embolization of the LAD results in a very high acute mortality in pigs (13). Repeated intracoronary injections of microspheres leads to significant reduction in LVEF (36%), but this model resembles more microvascular disease than coronary artery disease (2, 5). In addition to the fact that only distal coronaries can be targeted, another drawback of the acute occlusion models is that only little or no collateral formation occurs in these models.

The modern coronary angiography equipment used in this study enabled the assessment of collateral artery growth and LVEF during the follow-up, both of which are important endpoints in therapeutic trials. In the peripheral large animal models, X-ray and MR angiography have been used (1). However, in myocardial ischemia models, coronary collateral artery formation, i.e., the enlargement of the preexisting arterial anastomoses (also called arteriogenesis), has mostly been studied using postmortem angiography (4, 12, 19). We found that the complete occlusion of the target coronary vessel was required for induction of significant arteriogenesis, which corroborates the hypothesis that a significant pressure gradient must occur between the donor and the recipient arteries (19). In contrast, the continuous DAPT resulted in increased patency of the bottleneck stent, thus resulting in reduced collateral formation.

In some of the DAPT− animals with transmural infarction, we found that arteriogenesis was scarce possibly because these animals had sparse endogenous collateral anastomoses. In the dog model of intermittent 2-min coronary occlusions (8 times per day over 20 days) by a percutaneous pneumatic occluder, it was shown that the rate of collateral development was slower in dogs with poorer endogenous collaterals (7). Furthermore, the three repeated brief coronary occlusions by a balloon inflation appeared to recruit some collaterals in a porcine model but did not diminish ST-segment elevation (15), demonstrating that the preexisting collateral anastomoses need to enlarge over a few days to prevent transmural myocardial infarction in pigs. In our model, the few newly formed collaterals seen at 1 wk via angiography in pigs with transmural infarction were regressed by 4 wk, probably because infarcted myocardium needs only little blood flow, which corroborates the findings of a recent clinical study (3). An interesting finding in the study was that collateral growth was dynamic. First, the predominant phenomenon was the formation of collaterals from the contralateral side (from the RCA), with the later emphasis on collateral formation from the ipsilateral coronary artery, especially involving bridging collaterals. The latter phenomenon even led to regression of the contralateral RCA collaterals. Furthermore, arteriogenesis occurred mostly already during the first week after total occlusion, and little overall progression took place thereafter.

This study demonstrated that bottleneck stenting of the proximal LAD or LCX and discontinued DAPT caused ischemic cardiomyopathy with infarction area of 12–21% and decreased LVEF to 41–44% 4 wk after stenting with an acceptable mortality rate. The fact that most of the deaths occurred during the first week suggests that possible experimental therapies should usually be given 1–2 wk after the procedure in the one-vessel model. We also tested the feasibility of the two-vessel coronary artery stenosis model by implanting the bottleneck stents in both the proximal LCX and, 1 week later, in the mid-LAD, which to the best of our knowledge represents the first percutaneous two-vessel coronary artery stenosis model. The infarction area was smaller in this group than in either the LAD or LCX (DAPT−) groups, probably because of the continuous DAPT treatment preventing the early occlusion of the implanted stents and also because the second bottleneck stent was implanted in the mid LAD segment in contrast to the proximal LAD in the LAD-alone group. The two-vessel model led to significantly depressed LV function (LVEF 44% in survived animals), but the mortality rate was much higher (71%), indicating that occlusion of both branches of the LCA may not be applicable for therapeutic experiments in pigs.

In conclusion, the catheter-mediated approach for both chronic myocardial ischemia and ischemic heart failure created by the implantation of a bottleneck stent in the proximal LAD or LCX was shown to be feasible in domestic pigs. The bottleneck stent in the proximal LAD or LCX provides a novel porcine model of reversible myocardial ischemia, and the later occlusion of the stent leads to ischemic heart failure and collateral artery growth. This model can be used for the development of new therapeutic approaches. This study also gives important insight in the tempo-
rall spatial patterns of coronary collateral growth, and it demonstrates the feasibility of PET imaging for the assessment of potential therapeutic effects.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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H1308 THE BOTTLENECK STENT MODEL.