Letter to the Editor

Establishment of an experimental angiographic slow coronary flow model by microsphere embolism in swines

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No-reflow or slow-flow post revascularization remains a challenge in interventional cardiology. The “no-reflow” phenomenon is largely induced by microemboli of atherosclerotic debris, spasm, microvascular damage, and thrombi generated by percutaneous coronary intervention (PCI) procedure [1]. Clinical studies revealed that patients exhibiting no-reflow following reperfusion therapy for acute myocardial infarction (AMI) were associated with worse prognosis compared to patients without no-flow [1,2]. Currently, the mechanism of no-reflow has been exclusively studied in animal models of coronary artery ligation/reperfusion [5] and coronary microembolization (CME) [3–5] in canine or pig models, as well as in the rat coronary autologous coronary thrombotic microembolism model [6]. However, ischemia/reperfusion model in pig and canine only partly reflects pathological changes of no-reflow, since clinical “no-reflow” phenomenon is largely induced by microemboli [1] but not induced by coronary artery occlusion. Recently, Ma et al. reported that continuous injection of 120,000-42 μm microspheres into the left anterior descending (LAD) did not induce changes on coronary thrombolysis in myocardial infarction (TIMI) grade and TIMI 3 coronary flow was maintained immediately after microembolization and hemodynamic parameters remained stable before and after CME [7]. Till now, there is no large animal model presenting angiographic evidence of slow flow.

In this study, we established an angiographic coronary slow flow model in pig by repeated coronary injection of small doses of 40 μm microspheres. Eight male domestic pigs (3 to 4 months old, 25 ± 2 kg) were used in this study. Aspirin (2–3 mg/kg/d) was mixed in the food 3 days prior to experimental studies. All animals received humane care and that study protocols comply with the institution’s guidelines.

Pigs were anesthetized by an intramuscular injection of ketamine (15 mg/kg) combining with atropine (1 mg) and then fixed in a supine position on the workstation. 3–5 ml 3% pentobarbital sodium solution was injected via ear marginal vein on demand to maintain the anesthesia state. Oxygen saturation (SO2) was measured with pulse oximeter. Anticoagulation was induced with 200 IU/kg heparin sodium. The right femoral artery was dissected and a 6 F vascular sheath was placed for arterial access. After initial coronary angiography (CAG) using 6 F JR3.5 guiding catheter (Medtronic, Inc.), ventriculography and LV pressure measurements with 5 F Pigtail catheter (Cordis Inc.), a 2.6 F infusion microtubule catheter (Terumo Corporation) was then placed at the middle part of the LAD artery for microsphere injection, and 0.1 ml stock solution with 12,000 microspheres was diluted in 5 ml saline and injected for 20 s through the 2.6 F infusion microtubule catheter; this procedure was repeated, followed by 2 × 0.2 ml stock solution diluted into 5 ml saline with 24,000 microsphere injection, then 0.3 ml stock solution diluted into 5 ml saline with 36,000 microsphere injection (interval between each injection was 5 min) till the appearance of angiographic slow flow: TIMI frame count (TFC) > 40. LV pressures, ventriculography and CAG were measured at baseline and 3 h and catheters were withdrawn and animals were allowed to recover. All animals were fully recovered within 24 h and breathing spontaneously without intubation. One pig developed dyspnea (SO2 = 70%) during the procedure and recovered after 35 min mechanical respiration. Another pig died of cardiac failure at second day post procedure. Intramuscular analgesics (0.025 mg/kg IM butorphanol) were administered postoperatively as required to alleviate pain. Surviving animals (n = 7) were re-anesthetized at 4 weeks post procedure. Intramuscular analgesics (0.025 mg/kg IM butorphanol) were administered postoperatively as required to alleviate pain. Surviving animals (n = 7) were re-anesthetized at 4 weeks post procedure for hemodynamic and CAG examinations. LV end-diastolic volume and ejection fraction (LVEF) were assessed with quantitative ventriculography by an experienced investigator. The blood flow in the LAD was assessed with the TIMI scale and TFC (TIMI frame count) while myocardial tissue perfusion was analyzed with the TMPG scale (TIMI myocardial perfusion grade) [8]. Data were compared using the one-way analysis of variance (ANOVA). P < 0.05 were considered statistically significant. The mean injected stock solution was 2.45 ± 0.52 ml (around 294,000 microspheres) to induce slow flow in this model. Fig. 1 shows...
4 representative CAG imagines at baseline, immediately at the time of slow flow formation, 3 h and 4 weeks post microsphere injection. As shown in Table 1, TIMI flow was reduced to grade 2 at the time of slow flow formation and at 3 h post microsphere injection and TMPG reduced to grade 0.3 ± 0.5 at 3 h post microsphere injection, both recovered to grade 3 at 4 weeks post microsphere injection. TFC was significantly increased at 3 h post microsphere injection and returned to baseline level 4 weeks later. LVSP, dP/dtmax, dP/dtmin and LVEF were significantly reduced while LVEDP was significantly increased at 3 h and 4 weeks post procedure compared to baseline values (all p < 0.05). LV end-diastolic and end-systolic volumes were significantly increased at 4 weeks post injection compared to baseline level.

In this report, we presented angiographic slow flow evidence in pigs receiving repeated low-dose LAD microsphere injection. To our best knowledge, this is the first study reporting the angiographic evidence of slow flow in the large animal model.

The average of amount of LAD injected microspheres was about 294,000, which is 2 to 3 folds higher than previous reported amount used in the pig CME model without affecting TIMI grade (n = 120000)\textsuperscript{10}, suggesting that a larger amount of LAD microsphere injection is needed to induce slow flow phenomenon in the pig. Repeated low-dose LAD microsphere injections beginning with 12,000 microspheres (which was only the 1/10th dose used in the previous study\textsuperscript{10}) and followed by 24,000, then 36,000 microspheres are successful to induce angiographic slow flow. Repeated small amount LAD microsphere injections could be viewed as a kind of “ischemic preconditioning” [9], which might help to minimize the risk of developing malignant lethal arrhythmias and increase the tolerance of pigs to the larger amount of LAD microsphere injection needed to form angiographic slow flow. Despite the recovery of TIMI, TMPG and TFC values to baseline level, LV remodeling and dysfunction were still visible at 4 weeks post procedure, suggesting that this slow flow model mimicked to some extent the clinical situation of patients with no-flow post PCI procedure. Thus, this model might support investigation on underlying mechanisms and new therapy options for slow flow or no-flow.

Conflcit of interest

The authors report no relationships that could be construed as a conflict of interest.

\begin{table}
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\begin{tabular}{|l|c|c|c|}
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 & Baseline & 3 h & 4 weeks \\
\hline
TIMI (grade) & 3 ± 0 & 2 ± 0\textsuperscript{*} & 3 ± 0\textsuperscript{†} \\
TMPG (grade) & 3 ± 0 & 0.3 ± 0.5\textsuperscript{*} & 3 ± 0\textsuperscript{†} \\
TFC (frames) & 15.8 ± 3.4 & 29.2 ± 9.1\textsuperscript{*} & 30.2 ± 9\textsuperscript{†} \\
LVSP (mmHg) & 147 ± 15 & 113 ± 10\textsuperscript{*} & 123 ± 9\textsuperscript{†} \\
LVEDP (mmHg) & 7.8 ± 3.6 & 151 ± 2.0\textsuperscript{*} & 143 ± 1.2\textsuperscript{†} \\
HR (beats/min) & 129 ± 11 & 110 ± 7\textsuperscript{*} & 124 ± 6\textsuperscript{†} \\
dP/dtmax (mmHg/s) & 2887 ± 204 & 2156 ± 162\textsuperscript{*} & 2323 ± 209\textsuperscript{*} \\
dP/dtmin (mmHg/s) & −2417 ± 206 & −2081 ± 246\textsuperscript{*} & −2094 ± 334\textsuperscript{*} \\
LVEDV (ml) & 24.4 ± 3.3 & 25.3 ± 3.7 & 32.8 ± 4.2\textsuperscript{†} \\
LVESV (ml) & 7.7 ± 1.5 & 11.6 ± 2.6\textsuperscript{*} & 13.8 ± 3.0\textsuperscript{†} \\
LVEF (%) & 68 ± 5 & 55 ± 4\textsuperscript{*} & 58 ± 5\textsuperscript{†} \\
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\end{tabular}
\caption{Angiographic and hemodynamic parameters at baseline, 3 h and 4 weeks post LAD coronary microsphere injection.}
\end{table}
Acknowledgments

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References