PRE-CLINICAL RESEARCH

Intracoronary Poloxamer 188 Prevents Reperfusion Injury in a Porcine Model of ST-Segment Elevation Myocardial Infarction

Jason A. Bartos, MD, PhD,[a] Timothy R. Matsuura, BA,[b] Adamantios Tsangaris, MD,[a] Matthew Olson, MD,[a] Scott H. McKnite, BS,[a] Jennifer N. Rees, PhD,[a] Karen Haman, PhD,[c] Kadambari Chandra Shekar, MS,[b] Matthias L. Riess, MD, PhD,[d,e] Frank S. Bates, ScD,[c] Joseph M. Metzger, PhD,[b] Demetris Yannopoulos, MD[a]

VISUAL ABSTRACT

Bartos, J.A. et al. J Am Coll Cardiol Basic Trans Science. 2016;1(4):224–34.

ischemia and reperfusion. The CORE trial examined P188 for STEMI patients showing no benefit when it was infused through a peripheral IV catheter approximately 30 min after revascularization with thrombolytic therapy.

- STEMI was induced in pigs using endovascular coronary artery occlusion to compare intracoronary infusion of P188 immediately after revascularization to infusion of P188 through a peripheral IV catheter 30 min after revascularization. Immediate intracoronary infusion of vehicle control and PEG, a rheological control, were also compared.

- Intracoronary infusion of P188 immediately upon reperfusion reduced infarct size by 68% compared with delayed peripheral P188 infusion, which was similar to vehicle control.

- Mitochondrial respiration and calcium stress tolerance were preserved in the ischemic tissue of pigs treated with immediate intracoronary P188 infusion. Mitochondria from pigs with delayed peripheral P188 infusion were no different from control pigs.

- By reducing infarct size and mitochondrial dysfunction, immediate intracoronary infusion of P188 may provide a therapeutic strategy to improve post-STEMI outcomes. The timing and route of delivery were critical to the observed benefit.

HIGHLIGHTS

- STEMI remains a significant cause of in-hospital mortality, and up to 20% of people go on to develop heart failure.

- P188 is a nonionic triblock copolymer believed to prevent cellular injury after

SUMMARY

Poloxamer 188 (P188) is a nonionic triblock copolymer believed to prevent cellular injury after ischemia and reperfusion. This study compared intracoronary (IC) infusion of P188 immediately after reperfusion with delayed infusion through a peripheral intravenous catheter in a porcine model of ST-segment elevation myocardial infarction (STEMI). STEMI was induced in 55 pigs using 45 min of endovascular coronary artery occlusion. Pigs were then randomized to 4 groups: control, immediate IC P188, delayed peripheral P188, and polyethylene glycol infusion. Heart tissue was collected after 4 h of reperfusion. Assessment of mitochondrial function or infarct size was performed. Mitochondrial yield improved significantly with IC P188 treatment compared with control animals (0.25% vs. 0.13%), suggesting improved mitochondrial morphology and survival. Mitochondrial respiration and calcium retention were also significantly improved with immediate IC P188 compared with control animals (complex I respiratory control index: 7.4 vs. 3.7; calcium retention: 1,152 nmol vs. 386 nmol). This benefit was only observed with activation of complex I of the mitochondrial respiratory chain, suggesting a specific effect from ischemia and reperfusion on this complex. Infarct size and serum troponin I were significantly reduced by immediate IC P188 infusion (infarct size: 13.9% vs. 41.1%; troponin I: 19.2 μg/l vs. 77.4 μg/l). Delayed P188 and polyethylene glycol infusion did not provide a significant benefit. These results demonstrate that intracoronary infusion of P188 immediately upon reperfusion significantly reduces cellular and mitochondrial injury after ischemia and reperfusion in this clinically relevant porcine model of STEMI. The timing and route of delivery were critical to achieve the benefit. (J Am Coll Cardiol Basic Trans Science 2016;1:224–34) © 2016 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ABBREVIATIONS AND ACRONYMS

IC = intracoronary

IV = intravenous

P188 = poloxamer 188

PEG = polyethylene glycol

STEMI = ST-segment elevation myocardial infarction

Coronary heart disease accounts for 1 of 6 deaths each year in the United States, with an estimated 595,000 people experiencing myocardial infarction, of whom approximately 30% experience ST-segment elevation myocardial infarction (STEMI) (1). STEMI leads to in-hospital mortality in 6% of cases, and 13% to 20% of people go on to develop heart failure (2). STEMI-induced injury occurs in 2 stages. The first stage, ischemic injury, varies in severity depending on the pre-ischemic condition of the tissue, the duration of the ischemia, and the degree of revascularization. The second stage, reperfusion injury, is the injury initiated when blood flow returns to the ischemic tissue (3-5). Multiple cell injury pathways are thought to contribute to reperfusion injury, including membrane destabilization, dysregulation of calcium entry into the cell, free radical production, mitochondrial injury, and activation of pro-apoptotic pathways (6,7). Unfortunately, there is currently no clinical treatment available to prevent or reverse reperfusion injury.

Poloxamer 188 (P188) is a nonionic triblock co-polymer with a hydrophobic polyoxypropylene core flanked by hydrophilic polyoxyethylene segments (8,400 Da molecular weight; 0.17 PPO/PEO ratio). P188 has previously been shown to reduce red blood cell aggregation (8), facilitate clot lysis by recombinant tissue plasminogen activator (9), and reduce leukocyte chemotaxis (10). More recent studies have demonstrated that P188 stabilizes cellular membranes (11) and prevents lipid peroxidation induced by Fe^{2+} and H_2O_2 (12). Despite these effects, the CORE (Collaborative Organization for RheothRx Evaluation) trial found no clinical benefit of P188 when given to patients receiving thrombolytic therapy for STEMI (13). Subsequent decades have led to significant changes in

the standard of care for patients with STEMI, providing additional opportunities for treatment of myocardial injury.

We hypothesized that P188 would prevent reperfusion injury and reduce infarct size when infused immediately upon reperfusion with localized delivery into the coronary artery. We tested this in the clinically relevant setting of coronary angioplasty. We further hypothesized that recreating the treatment conditions of the CORE trial with delayed infusion of the same dose of P188 through a peripheral intravenous (IV) catheter would demonstrate no benefit.

FIGURE 1 Description of the Experimental Groups

(A) A schematic representation of the experimental protocols. Infarction was produced with 45 min of endovascular coronary occlusion. Treatment began at reperfusion in the control, P188, and PEG groups with intracoronary (IC) infusion of saline (1.7 ml/kg), P188 (250 mg/kg), or PEG (250 mg/kg), respectively, over 30 min. The delayed P188 group received intravenous (IV) P188 infusion (250 mg/kg) beginning 30 min after reperfusion began. All groups received an intravenous maintenance infusion of the respective therapy (1.7 ml/kg saline control or 250 mg/kg for all others) lasting 3 h. Hearts were harvested at 4 h post-reperfusion for further analysis. **(B)** The sample size is shown for each treatment group as divided into the mitochondrial and infarct size analysis groups.
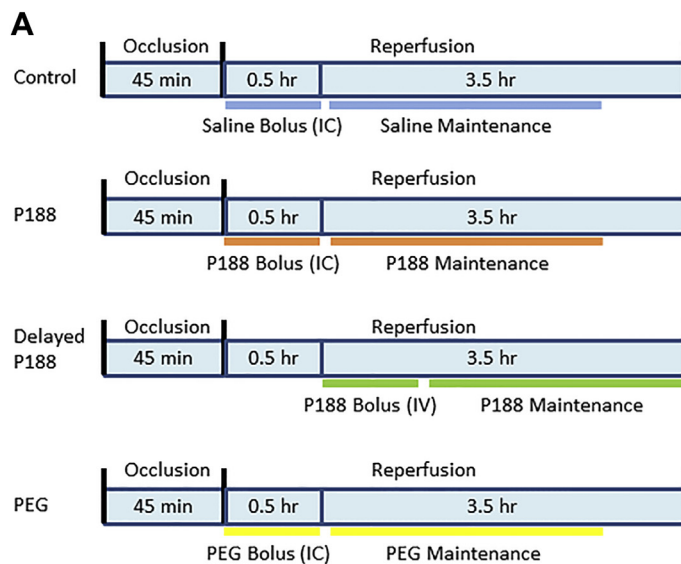
METHODS

All studies were performed with approval from the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation and the National Research Council's 1996 Guidelines for the Care and Use of Laboratory Animals (Protocol 15-02-03).

ANIMAL MODEL. Yorkshire pigs, 38 to 42 kg, were used for all studies. The anesthesia, surgical preparation, and data monitoring and recording were performed as described in detail previously (14). Briefly, pigs were anesthetized with ketamine (10 ml of 100 mg/ml) followed by inhaled isoflurane at a dose of 0.8% to 1.2% for the remainder of the study. Animals were intubated with a 7.0-mm endotracheal tube. Temperature was measured with an esophageal temperature probe and was maintained at 37.5 ± 0.5°C with a warming blanket throughout the study. Vascular access was obtained in the bilateral femoral arteries with 8-F sheaths placed percutaneously under ultrasound guidance. An 8-F sheath was placed in the right external jugular vein. A right ear vein IV catheter was placed. The central aortic pressure was monitored continuously with a micromanometer-tipped catheter (Millar Instruments, Houston, Texas) placed through the left femoral sheath, and the right atrial pressure was measured with a second micromanometer-tipped catheter placed in the right external jugular sheath. All animals received an IV heparin bolus (100 U/kg) and 1,000 U heparin every 2 h thereafter. The animals were ventilated with room air using volume-controlled ventilation (Narkomed, Draeger Medical, Telford, Pennsylvania) with tidal volume of 10 ml/kg and a respiratory rate adjusted to maintain $PaCO_2$ of 40 mm Hg and arterial O_2 saturation >95% as measured from arterial blood (Gem 300, Instrumentation Laboratory, Bedford, Massachusetts). Surface electrocardiographic tracings were continuously recorded. All data were recorded with a BIOPAC digital acquisition system (BIOPAC Systems Inc., Goleta, California).

EXPERIMENTAL PROTOCOLS. Animals (n = 55) were randomized into 1 of 4 treatment groups: control, P188, delayed P188, and polyethylene glycol (PEG) 8,000 Da. Each treatment group was further divided into 2 groups: mitochondrial studies or assessment of infarct size (**Figure 1**). The protocol details were described previously (15) and are described briefly in the following text.

Blood viscosity assessment. Arterial blood was collected from animals prior to ischemia. Heparin was added (10 U/ml) to prevent thrombus formation. Hematocrit was measured at 28%. Blood was added to

an AR-G2 rheometer with a couette attachment (TA Instruments, New Castle, Delaware). Viscosity was assessed at 37°C with varied shear rate ranging from 0.1 to 100 s^{-1}. Measurements were repeated at various concentrations of P188 and PEG.

Percutaneous coronary occlusion. The left main coronary artery was engaged with a 6-F AL 0.75 guide (Cordis, Fremont, California) advanced from the right femoral sheath with fluoroscopic guidance (OEC 9800, General Electric, Fairfield, Connecticut). Coronary angiography was performed with injection of Omnipaque contrast (GE Healthcare, Dublin, Ireland). For mitochondrial studies, a 0.014-inch guidewire was passed through the left circumflex artery into the second obtuse marginal branch, over which a 2.75 × 8 mm monorail bare-metal stent (Abbott Vascular, Abbott Park, Illinois) was inserted. The balloon was inflated, thereby deploying the stent and occluding the vessel. For infarct size studies, a 0.014-inch guidewire was inserted into the left anterior descending (LAD) artery and a 2.75 × 8 mm monorail balloon (Medtronic, Minneapolis, Minnesota) was advanced distal to the second diagonal branch. The balloon was inflated occluding flow but allowing flow to the second diagonal.

In all studies, vessel occlusion was verified with intracoronary (IC) contrast injection showing no passage beyond the balloon. The guide was disengaged from the left main coronary artery to prevent restriction of flow to the entire left coronary circulation. The vessel remained occluded for 45 min. The animal was then treated with 40 mg amiodarone IV, the guide was reinserted into the left main coronary artery, and the balloon was deflated and removed. A brief coronary angiogram was performed to verify coronary blood flow to the ischemic territory. P188, PEG, or saline vehicle control were delivered as indicated (**Figure 1**).

Arrhythmia, including ventricular tachycardia and ventricular fibrillation, were treated with defibrillation as needed. All animals achieved return of spontaneous circulation and completed 4 h of reperfusion time.

Isolation of mitochondria. After 4 h of reperfusion, animals were euthanized and the heart was removed. Tissue was excised from the area of the second obtuse marginal branch distal to the stent (infarct) as well as the area adjacent to the LAD (nonischemic).
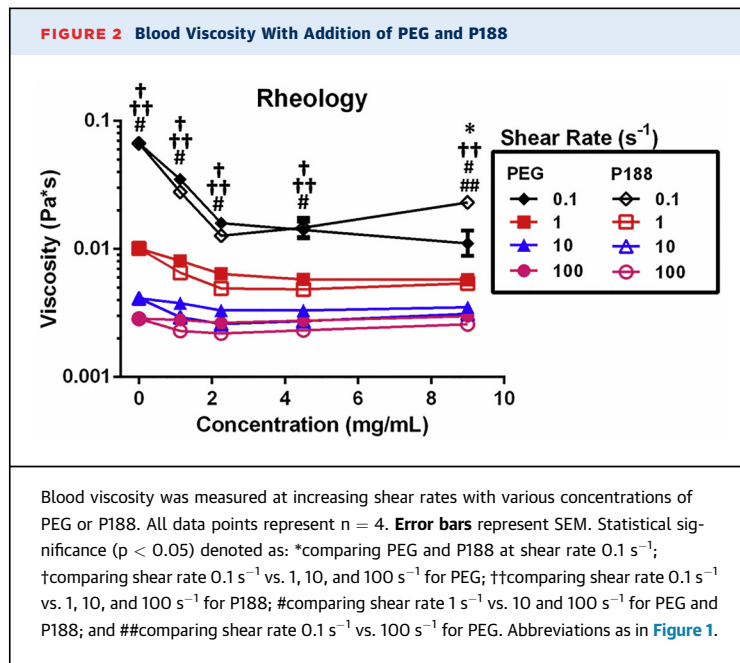
Mitochondrial experiments were performed as described previously (16). Briefly, excised cardiac tissue was immediately placed in ice cold isolation buffer containing 200 mmol/l mannitol, 50 mmol/l sucrose, 5 mmol/l KH$_2$PO$_4$, 5 mmol/l 3-(n-morpholino) propanesulfonic acid (MOPS), 1 mmol/l ethylene glycol tetraacetic acid, and 0.1% bovine serum albumin

(BSA), pH 7.15. The tissue was then manually minced and homogenized with a blade homogenizer (CAT Scientific, Paso Robles, California) in the presence of 5 U/ml protease (*Bacillus licheniformis*). The protease was then diluted 10-fold, and homogenization was repeated. Mitochondria were isolated by differential centrifugation including 10 min at 8,000 × *g* followed by resuspension of the pellet and centrifugation at 750 × *g* for 10 min to remove cellular debris. Isolated mitochondria were quantified by Bradford protein quantification assay (Sigma, St. Louis, Missouri). The mitochondrial yield was calculated as the ratio of total protein mass of isolated mitochondrial to total protein mass of the excised heart tissue from which they were isolated.

Mitochondrial respiration. Mitochondrial oxygen consumption was measured at 25°C using a Clark-type O$_2$ electrode (Model 1302, Strathkelvin Instruments, North Lanarkshire, Scotland) in a water-jacketed chamber. Mitochondria (275 μg) were added to the respiration chamber with experimental buffer containing 130 mmol/l KCl, 5 mmol/l K$_2$HPO$_4$, 20 mmol/l MOPS, and 0.1% BSA, pH 7.15. State 2 respiration was initiated with the addition of substrate specific for either complex I (10 mmol/l pyruvate/malate) or complex II (10 mmol/l succinate and 10 mmol/l rotenone, a complex I blocker) of the mitochondrial respiratory chain. State 3 respiration then began with addition of 250 mmol/l adenosine diphosphate. State 4 was observed when the phosphorylation of adenosine diphosphate to adenosine triphosphate was complete. The respiratory control index (RCI) was calculated as the ratio of state 3 to 4 respiration.

Mitochondrial calcium retention. Mitochondria (0.5 mg/ml) were combined with complex I (10 mmol/l pyruvate/malate) or complex II (10 mmol/l succinate and 10 μmol/l rotenone) substrates and buffer containing 130 mmol/l KCl, 5 mmol/l K$_2$HPO$_4$, 20 mmol/l MOPS, and 0.1% BSA, pH 7.15. CaGreen-5N hexapotassium salt (100 nmol/l) was added to monitor extra-mitochondrial calcium at excitation 510 nm and emission 531 nm (Life Technologies, Grand Island, New York). CaCl$_2$ (5 mmol/l) was infused at a rate of 150 nmol/min until the CaGreen fluorescence reached steady state. A sudden increase in CaGreen emission suggested an opening of the mitochondrial permeability transition pore (mPTP). The calcium retention capacity is quantified as the calcium required to trigger mPTP opening.

Infarct size assessment. After 4 h of reperfusion, the left main coronary artery was again engaged with a 6-F AL 0.75 guide. The right coronary artery was engaged with a 6-F hockey stick guide. The 0.014-inch guidewire was again advanced into the LAD,

FIGURE 2 Blood Viscosity With Addition of PEG and P188

Blood viscosity was measured at increasing shear rates with various concentrations of PEG or P188. All data points represent n = 4. **Error bars** represent SEM. Statistical significance (p < 0.05) denoted as: *comparing PEG and P188 at shear rate 0.1 s^{-1}; †comparing shear rate 0.1 s^{-1} vs. 1, 10, and 100 s^{-1} for PEG; ††comparing shear rate 0.1 s^{-1} vs. 1, 10, and 100 s^{-1} for P188; #comparing shear rate 1 s^{-1} vs. 10 and 100 s^{-1} for PEG and P188; and ##comparing shear rate 0.1 s^{-1} vs. 100 s^{-1} for PEG. Abbreviations as in **Figure 1**.

over which a 3.0 × 8 mm over-the-wire balloon was inserted distal to the second diagonal branch in the same position as the prior balloon as verified by fluoroscopy. The balloon was inflated, the guidewire was removed, and 40 ml of 2% 2,3,5 triphenyltetrazolium chloride was infused through the lumen of the balloon catheter. Subsequently, 60 ml of 1% Evans Blue dye (Sigma) was infused in each of the 2 guides perfusing the left main coronary artery and right coronary artery simultaneously. These infusions induced ventricular fibrillation. The heart was then excised and sliced in 1-cm-thick slices from the apex to the base of the heart. Each side of the slice was photographed. The infarct (white) and area at risk (red) were quantified using ImageJ (National Institutes of Health, Bethesda, Maryland).

Troponin and creatinine assessment. Arterial blood was collected prior to infarction and after 4 h of reperfusion. Cardiac-specific troponin I (cTnI) was quantified using a 2-site sandwich assay (Stratus CS Acute Care, Siemens, Munich, Germany). Creatinine was measured with standard human assays. Personnel performing the analysis were blinded to the treatment.

STATISTICAL ANALYSIS. Values are expressed as mean ± SE of the mean. All statistics were performed with GraphPad Prism 6 (GraphPad Software, La Jolla, California). The data were normality tested with the D'Agostino-Pearson omnibus test indicating that all datasets were sufficiently normally distributed. Rheology data were compared between shear rates using a repeated measures analysis of variance with the Fisher Least Significant Difference post-hoc

analysis. Comparisons of mitochondrial yield, RCI, and calcium retention capacity between infarct and nonischemic tissues were performed using paired Student *t* tests with multiplicity adjustment using the Holm-Sidak method. All other data were compared using a 1- or 2-way analysis of variance as appropriate and Fisher Least Significant Difference post-hoc analysis. A 2-tailed p value <0.05 was considered statistically significant.

RESULTS

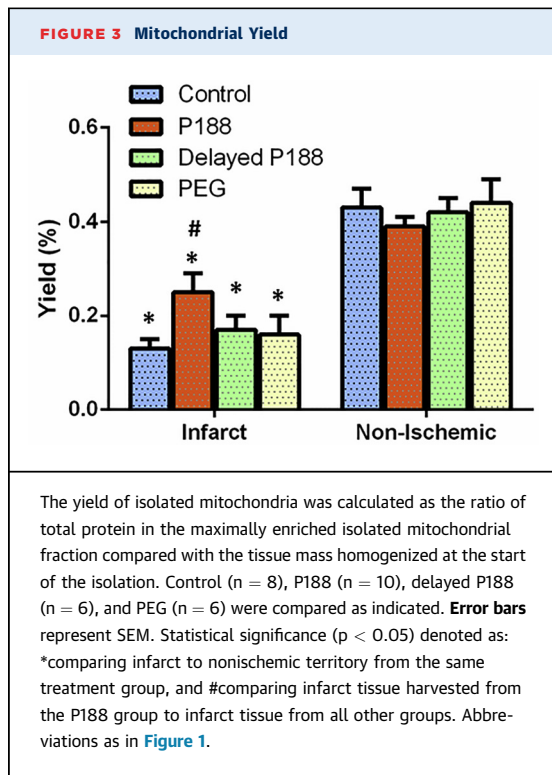### INFLUENCE OF P188 AND PEG ON BLOOD VISCOSITY.
PEG was chosen as a viscosity control for P188 on the basis of molecular weight and similar composition; however, the effect of PEG on blood viscosity was not well described. As shown in **Figure 2**, the addition of increasing concentrations of PEG and P188 resulted in similar reductions in whole blood viscosity. Increasing the shear rate reduced the whole blood viscosity independent of P188 and PEG, partially negating the effect of PEG and P188. At low shear rates, high-dose P188 (9 mg/ml) reduced viscosity to a modestly lesser degree compared with PEG (0.023 Pa·s vs. 0.011 Pa·s; p < 0.001). The viscosity effects of P188 and PEG were otherwise similar.

MITOCHONDRIAL YIELD IMPROVES WITH P188 TREATMENT.
Isolation of mitochondria by differential centrifugation requires intact mitochondria of stable density. Therefore, reductions in mitochondrial yield suggest loss of membrane integrity (i.e., rupture) or substantial change in morphology (i.e., swelling and destabilization). Although mitochondrial yield was reduced in the infarct territory of all groups, IC P188 significantly improved the yield of mitochondria isolated compared with control animals (0.25% vs. 0.13%; p = 0.012) (**Figure 3**). In contrast, delayed P188 demonstrated no significant improvement over control animals (0.17% vs. 0.13%; p = 0.45). Intracoronary PEG demonstrated no significant difference compared with control animals (0.16% vs. 0.13%; p = 0.57).

MITOCHONDRIAL FUNCTION IS PRESERVED WITH P188 TREATMENT.
The central role of mitochondria is respiration resulting in synthesis of adenosine triphosphate. The RCI using substrates specific for complex I of the mitochondrial respiratory chain was significantly lower in infarct tissue from control animals, delayed P188, and PEG animals compared with noninfarct tissue (3.7 vs. 8.2, 3.9 vs. 8.2, 4.2 vs. 9.3, respectively; p < 0.001) (**Figure 4A**). In contrast, the complex I RCI of infarct tissue from P188-treated animals was significantly higher compared with control animals (7.4 vs. 3.7; p < 0.001). Further, it was not statistically different compared with nonischemic

FIGURE 3 Mitochondrial Yield

The yield of isolated mitochondria was calculated as the ratio of total protein in the maximally enriched isolated mitochondrial fraction compared with the tissue mass homogenized at the start of the isolation. Control (n = 8), P188 (n = 10), delayed P188 (n = 6), and PEG (n = 6) were compared as indicated. **Error bars** represent SEM. Statistical significance (p < 0.05) denoted as: *comparing infarct to nonischemic territory from the same treatment group, and #comparing infarct tissue harvested from the P188 group to infarct tissue from all other groups. Abbreviations as in **Figure 1**.

Complex II RCI was significantly reduced for mitochondria isolated from infarct tissue in all treatment groups when compared with nonischemic tissue (2.1 vs. 3.4, 2.7 vs. 3.5, 2.2 vs. 3.9, 2.3 vs. 3.8 for control, P188, delayed P188, and PEG, respectively; p < 0.01) (**Figure 4B**). There were no significant differences between the treatment groups when comparing ischemic tissues or nonischemic tissues.

Calcium retention, a measure of mitochondrial membrane integrity and mitochondrial function, was significantly reduced in the infarct tissue of all groups in the presence of complex I substrates when compared with nonischemic tissue (**Figure 5A**). However, calcium retention of mitochondria isolated from P188-treated animals was significantly preserved compared with control, delayed P188, and PEG animals (1,152 nmol vs. 386, 451, and 423 nmol, respectively; p < 0.001).

In the setting of complex II substrates, mitochondria isolated from the infarct territory of P188-treated animals continued to tolerate higher levels of calcium compared with control, delayed P188, and PEG animals (1,481 nmol vs. 848, 843, and 881 nmol, respectively; p < 0.005) (**Figure 5B**). However, mitochondria from infarct tissue from P188-treated animals also retained significantly greater levels of calcium than the nonischemic mitochondria from P188-treated animals (1,481 vs. 1,005 nmol; p = 0.019). There were no significant differences in calcium retention capacity of the mitochondria taken from control, delayed P188, and PEG animals when infarct and nonischemic tissues were compared.
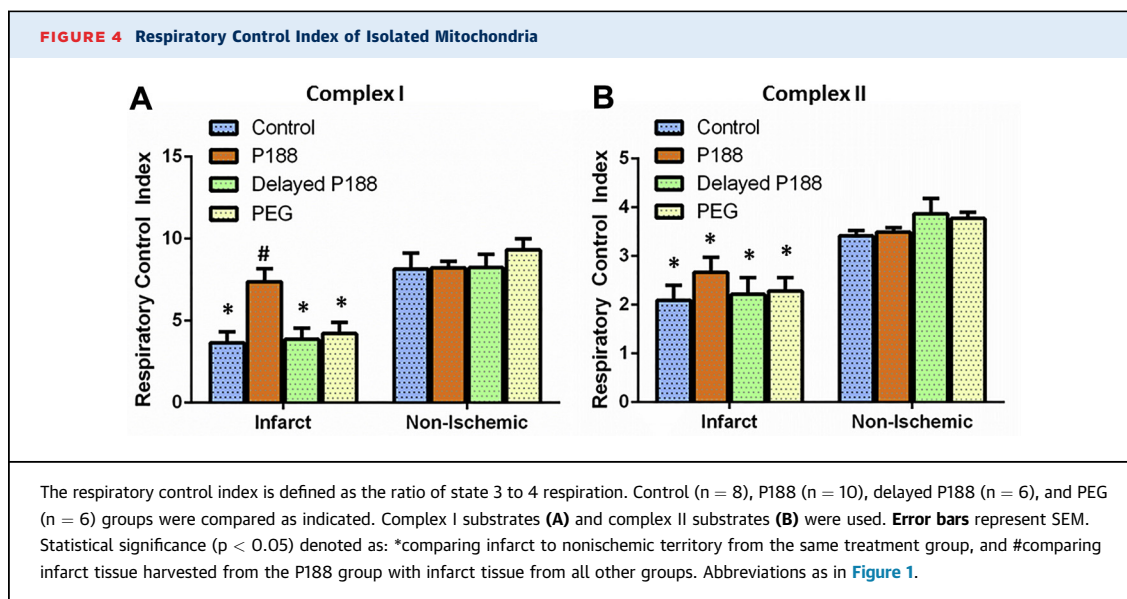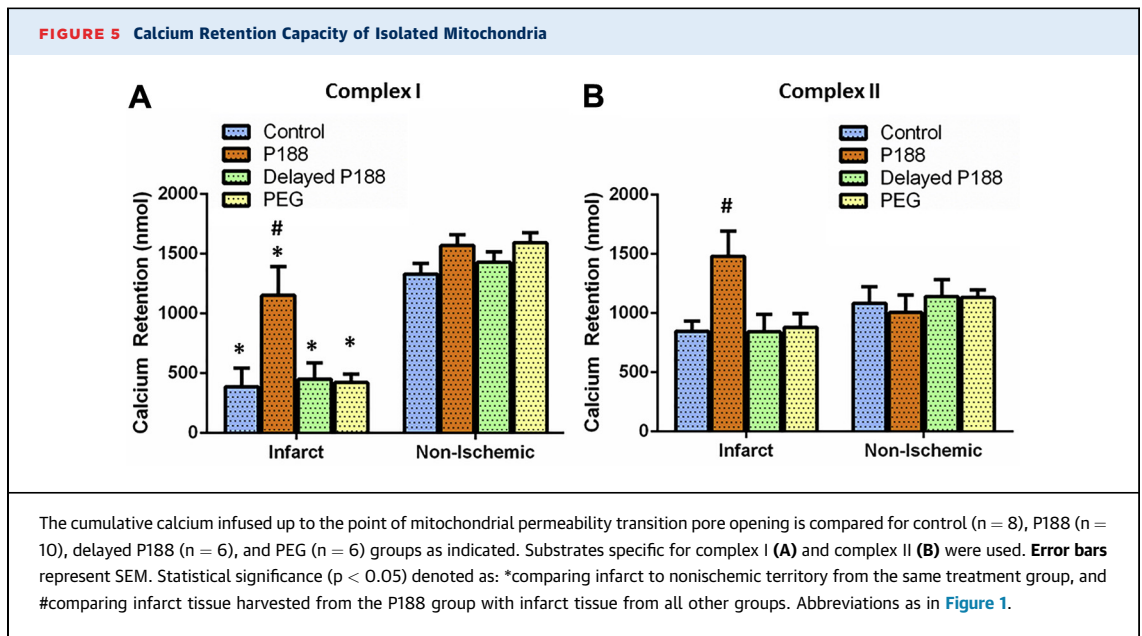
tissue (7.4 vs. 8.2; p = 0.85), suggesting near total preservation of mitochondrial respiration through complex I. Complex I RCI for infarct tissue from delayed P188 and PEG-treated animals were similar to infarct tissue from control animals (3.9 and 4.2 vs. 3.7 for delayed P188 [p = 0.85] and PEG [p = 0.61], respectively).

FIGURE 4 Respiratory Control Index of Isolated Mitochondria

The respiratory control index is defined as the ratio of state 3 to 4 respiration. Control (n = 8), P188 (n = 10), delayed P188 (n = 6), and PEG (n = 6) groups were compared as indicated. Complex I substrates **(A)** and complex II substrates **(B)** were used. **Error bars** represent SEM. Statistical significance (p < 0.05) denoted as: *comparing infarct to nonischemic territory from the same treatment group, and #comparing infarct tissue harvested from the P188 group with infarct tissue from all other groups. Abbreviations as in **Figure 1**.

FIGURE 5 Calcium Retention Capacity of Isolated Mitochondria

The cumulative calcium infused up to the point of mitochondrial permeability transition pore opening is compared for control (n = 8), P188 (n = 10), delayed P188 (n = 6), and PEG (n = 6) groups as indicated. Substrates specific for complex I **(A)** and complex II **(B)** were used. **Error bars** represent SEM. Statistical significance (p < 0.05) denoted as: *comparing infarct to nonischemic territory from the same treatment group, and #comparing infarct tissue harvested from the P188 group with infarct tissue from all other groups. Abbreviations as in **Figure 1**.

INFARCT SIZE AND TROPONIN RELEASE ARE REDUCED WITH P188 TREATMENT. Infarct size, as a percentage of area at risk, was significantly reduced with immediate IC P188 infusion compared with control, delayed P188, and PEG treatment animals (13.9% vs. 41.1%, 42.8%, and 45.6%, respectively; p < 0.001) (**Figures 6A to 6D**). The infarct size with control, delayed P188, and PEG treatments were not significantly different. Serum cTnI was significantly reduced at 4 h in the P188-treated animals as compared with the control, delayed P188, and PEG groups (19.2 μg/l vs. 77.4, 68.8, and 65.1 μg/l, respectively; p < 0.05) (**Figure 6E**). There were no significant differences between the control, delayed P188, and PEG groups.

SAFETY ENDPOINTS. Ventricular arrhythmias including ventricular tachycardia and ventricular fibrillation occurred in 18% of animals overall. Of the ventricular arrhythmias, 40% developed during ischemia, whereas 60% occurred during reperfusion. There were no significant differences between treatment groups. All animals achieved return of spontaneous circulation and completed the 4-h reperfusion time. Angiography was performed immediately after deflation of the coronary balloon and before medication infusion, demonstrating Thrombolysis In Myocardial Infarction flow grade 3 and similar myocardial blush in all animals. There were no significant differences in creatinine levels between groups at baseline or 4 h after reperfusion. The 4-h creatinine values for control, P188, delayed P188, and PEG groups were 1.1, 1.2, 0.96, and 1.1 mg/dl, respectively.

DISCUSSION

This study reports a significant reduction in cardiac injury with decreased infarct size and preserved mitochondrial function after STEMI using a first-in-class membrane stabilizing block copolymer called P188. These findings highlight the importance of copolymer delivery route and timing to achieve meaningful myocardial preservation in reperfusion, as we find that immediate and direct IC infusion, but not delayed peripheral delivery, is essential to prevent myocardial injury. These findings may help explain the negative outcomes of the CORE trial, which applied a delayed and peripheral delivery strategy in an era when thrombolysis was widely used.

Previous studies have proposed 2 distinct mechanisms for the effects of P188. The first, altered blood viscosity leading to improved tissue perfusion, is not the primary mechanism, as PEG infusion fails to provide a similar benefit despite similar viscosity effects. The second proposed mechanism involves reduction in reperfusion injury through stabilization of sarcolemmal membranes damaged by the metabolic derangements of ischemia (11,12). Although this mechanism was not tested directly in the current study, it may be supported by the improvement in mitochondrial calcium retention capacity observed. Direct effects of P188 on mitochondria in situ have not been shown in prior studies and are beyond the scope of the current study. Further studies will be required to elucidate the specific mechanism of the benefit.

FIGURE 6 Infarct Size
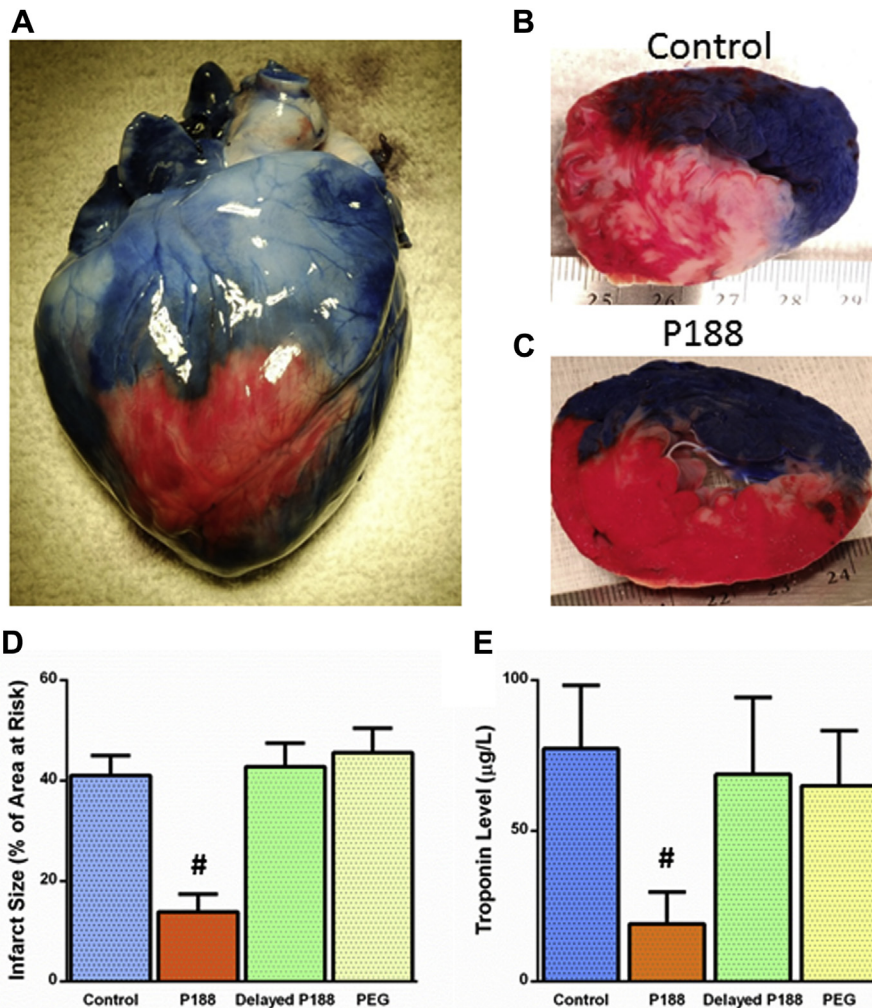
(A) Hearts were stained in vivo with 2,3,5 triphenyltetrazolium chloride and Evans Blue dye. The hearts were sliced in 1-cm slices revealing infarct **(white)**, area at risk **(red)**, and nonischemic tissue **(blue)**. Representative slices for control **(B)** and P188 **(C)** animals are shown. Infarct size as a percent of area at risk in all slices from control (n = 6), P188 (n = 7), delayed P188 (n = 6), and PEG (n = 6) groups is quantified **(D)**. Serum troponin levels at 4 h of reperfusion were quantified **(E)**. **Error bars** represent SEM. #Statistical significance (p < 0.05) comparing the P188 group to all other groups.

Ischemia-reperfusion has previously been shown to reduce mitochondrial respiration predominantly at complex I in agreement with the current findings (17). This down-regulation may be due to damage incurred by the respiratory complex, or it may be a result of controlled inhibition of complex I activity possibly reducing the formation of reactive oxygen species that can occur at complex I (18). This study demonstrates near normalization of complex I activity with P188 treatment. The significant improvement in mitochondrial yield further supports the hypothesis that mitochondrial health is improved with P188

treatment. Although serving as a marker of mitochondrial health, preserved complex I function may also provide significant bioenergetic benefits for mitochondria experiencing the severe stress of ischemia-reperfusion.

Uncontrolled calcium influx during ischemia-reperfusion presents a severe challenge to the mitochondria with calcium overload leading to mPTP opening, loss of membrane potential, cessation of respiration, and release of pro-apoptotic factors. Immediate IC P188 treatment demonstrated significant improvement in calcium retention in the infarct

TABLE 1 Comparison of the CORE Trial and the Current Study	
CORE Trial	**Current Study**
Human patients with STEMI	Translational study with a porcine STEMI model
Thrombolytic therapy	Primary angioplasty
Reperfusion in 54% of patients	100% reperfusion
P188 infused via peripheral IV P188 delivered ~30 min after reperfusion	Intracoronary P188 infusion P188 infused immediately upon reperfusion
No clinical benefit observed	66% reduction in infarct size Preserved mitochondrial function
Renal dysfunction in P188-treated patients	No renal dysfunction early after treatment with purified P188

CORE = Collaborative Organization for RheothRx Evaluation.

tissue without any significant effect in the non-ischemic tissue. The mechanism of improved calcium retention is unclear, but it may indicate that P188 is preventing mitochondrial calcium loading in an injury-dependent manner. Importantly, the group receiving delayed peripheral P188 infusion did not demonstrate any of these benefits, suggesting that early and direct infusion was critically important.

The CORE trial, a phase II, open-label clinical trial published in 1997, tested the safety and potential benefits of P188 in 2,948 STEMI patients receiving thrombolytic therapy (13). No clinical benefit was observed. However, multiple significant limitations to the protocol can now be observed (**Table 1**). First, P188 was delivered by peripheral IV approximately 30 min after thrombolytic therapy was administered. Although the detailed timing is not described in the published study, the CORE trial protocol recommended randomization within 15 min of thrombolytic infusion with initiation of the P188 within 15 min of randomization. This provides a 30-min delay in P188 therapy from the time of revascularization. Prior studies using cyclosporine, known to prevent reperfusion injury by inhibiting opening of the mPTP in the setting of cardiac arrest, have shown a severe degradation in efficacy of treatment within as little as 3 min after return of spontaneous circulation (19). Therefore, the 30-min delay in the CORE trial may have negated the opportunity to attenuate reperfusion injury. In contrast, our study demonstrates a 68% reduction in infarct size when a similar dose of P188 was delivered directly into the affected coronary artery immediately after reperfusion.

Use of primary percutaneous coronary intervention (PCI) in the current study represents the current standard of care for STEMI patients in the United States where catheterization laboratories are available. Until recently, thrombolytic therapy was the standard treatment modality for patients experiencing myocardial infarction. However, 26% of patients failed to achieve reperfusion even in recent trials (20). In the CORE trial, which exclusively enrolled patients receiving thrombolytic therapy, 46% of control subjects did not achieve revascularization after administration of the thrombolytic. If reperfusion is never achieved, treatments that prevent reperfusion injury will be ineffective. PCI has been shown to result in normal coronary artery flow in over 90% of patients (20). Although absolutely necessary, reinitiation of coronary flow increases the potential for reperfusion injury, creating a need for innovative therapies to prevent this damage (4).

Although myocardial infarction is the most common cause of ischemia/reperfusion injury, other medical scenarios trigger similar pathology, including organ transplantation, cardiac surgery using cardioplegia, and cardiac arrest. Other organ systems can also be affected in situations of stroke or cardiac arrest. P188 was recently tested in combination with other pharmacological and mechanical treatments for ischemia/reperfusion injury in a porcine model of prolonged ventricular fibrillation cardiac arrest, where it showed considerable improvement in cardiac function and neurologically intact survival (21). P188 has also shown a direct benefit in neurons exposed to ischemia with prevention of apoptosis and preservation of the blood-brain barrier (22,23).

The CORE trial discovered a significant reduction in the renal function of patients receiving P188. This was particularly prominent in elderly patients and in those with pre-existing renal disease. Subsequent studies have demonstrated a reduction in the renal effects of P188 when the compound is purified to reduce low molecular weight contaminants (24). The P188 used in our study was highly purified in its commercial form, presumably limiting the renal effects. No difference was observed in creatinine after 4 h of treatment, although renal dysfunction may not materialize until later in the course and must be studied further.

STUDY LIMITATIONS. First, this study does not elucidate a clear mechanism of action for P188. Further, although the dose was chosen on the basis of that used in prior animal studies and the CORE trial, dose optimization was not undertaken. It is clear from our study that timing and route of delivery have a significant effect on outcomes, possibly due to the higher effective dose when delivered directly into the coronary arteries. Although this suggests that a significantly higher systemic dose may provide a similar benefit if delivered early in reperfusion, potential side effects of treatment may also become more prominent.

Important differences also remain when comparing this pre-clinical porcine model system to human patients. First, this model requires the presence of inhaled anesthetic to maintain animal comfort. This would be expected to mask the effects of P188, as the inhaled anesthetic itself may have pre- and post-conditioning benefits in all animal groups studied (16,25). However, this would be clinically relevant in the setting of cardiac surgery and transplant. The demonstrated benefit with P188 may, therefore, be an underestimate of its true benefit in the typical STEMI patient population. In addition, the animals do not have underlying comorbidities, which often accompany coronary artery disease and may alter the benefits of P188.

Last, this study is limited in the duration of follow-up, with up to 4 h of monitoring post-P188. Further studies with longer follow-up would need to be performed to assess renal function and cardiac benefit. These studies would not be expected to demonstrate a significant reduction in benefit, as the early predictors of infarct size and cTnI release are well-established to correlate with long-term outcomes.

CONCLUSIONS

This study demonstrates a substantial cardiac benefit of P188 after STEMI using a clinically relevant treatment strategy of IC infusion of P188 at the time of reperfusion. The model is clinically relevant, incorporating PCI similar to the current standard of care in human patients. The results demonstrate that early IC infusion is critical to achieving the benefit.

REPRINT REQUESTS AND CORRESPONDENCE: Dr. Demetris Yannopoulos, Cardiology Division, University of Minnesota, 420 Delaware Street SE, MMC 508, Minneapolis, Minnesota 55455. E-mail: yanno001@umn.edu.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: STEMI causes significant morbidity and mortality. Reperfusion injury, the injury induced by reconstitution of blood flow into the ischemic area, accounts for as much as 50% of the myocardial injury. Treatment at the time of reperfusion provides an opportunity to prevent this injury.

TRANSLATIONAL OUTLOOK: Although the mechanism remains unknown, P188 provides a significant reduction in reperfusion injury in this porcine model of STEMI. Further studies are required to assess the long-term outcomes after P188 treatment in animals. Subsequent studies should focus on human trials.

REFERENCES

1. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics—2014 update: a report from the American Heart Association. Circulation 2014;129:e28–292.

2. Kontos MC, Rennyson SL, Chen AY, Alexander KP, Peterson ED, Roe MT. The association of myocardial infarction process of care measures and in-hospital mortality: a report from the NCDR(R). Am Heart J 2014;168:766–75.

3. Ovize M, Baxter GF, Di Lisa F, et al. Post-conditioning and protection from reperfusion injury: where do we stand? Position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. Cardiovasc Res 2010;87:406–23.

4. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. N Engl J Med 2007;357:1121–35.

5. Ibanez B, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. J Am Coll Cardiol 2015;65:1454–71.

6. Bartos JA, Debaty G, Matsuura T, Yannopoulos D. Post-conditioning to improve cardiopulmonary resuscitation. Curr Opin Crit Care 2014;20:242–9.

7. Tsang A, Hausenloy DJ, Yellon DM. Myocardial postconditioning: reperfusion injury revisited. Am J Physiol Heart Circ Physiol 2005;289:H2–7.

8. Adams-Graves P, Kedar A, Koshy M, et al. RheothRx (poloxamer 188) injection for the acute painful episode of sickle cell disease: a pilot study. Blood 1997;90:2041–6.

9. Carr ME Jr., Powers PL, Jones MR. Effects of poloxamer 188 on the assembly, structure and dissolution of fibrin clots. Thromb Haemost 1991;66:565–8.

10. Schaer GL, Hursey TL, Abrahams SL, et al. Reduction in reperfusion-induced myocardial necrosis in dogs by RheothRx injection (poloxamer 188 N.F.), a hemorheological agent that alters neutrophil function. Circulation 1994;90:2964–75.

11. Martindale JJ, Metzger JM. Uncoupling of increased cellular oxidative stress and myocardial ischemia reperfusion injury by directed sarcolemma stabilization. J Mol Cell Cardiol 2014;67:26–37.

12. Marks JD, Pan CY, Bushell T, Cromie W, Lee RC. Amphiphilic, tri-block copolymers provide potent membrane-targeted neuroprotection. FASEB J 2001;15:1107–9.

13. Collaborative Organization for RheothRx Evaluation (CORE). Effects of RheothRx on mortality, morbidity, left ventricular function, and infarct size in patients with acute myocardial infarction. Circulation 1997;96:192–201.

14. Segal N, Matsuura T, Caldwell E, et al. Ischemic postconditioning at the initiation of cardiopulmonary resuscitation facilitates functional cardiac and cerebral recovery after prolonged untreated ventricular fibrillation. Resuscitation 2012;83:1397–403.

15. Yannopoulos D, Zviman M, Castro V, et al. Intra-cardiopulmonary resuscitation hypothermia with and without volume loading in an ischemic model of cardiac arrest. Circulation 2009;120:1426–35.

16. Riess ML, Matsuura TR, Bartos JA, et al. Anaesthetic postconditioning at the initiation of CPR improves myocardial and mitochondrial function in a pig model of prolonged untreated ventricular fibrillation. Resuscitation 2014;85:1745–51.

17. Xu A, Szczepanek K, Hu Y, Lesnefsky EJ, Chen Q. Cardioprotection by modulation of mitochondrial respiration during ischemia-reperfusion: role of apoptosis-inducing factor. Biochem Biophys Res Commun 2013;435:627–33.

18. Chouchani ET, Pell VR, Gaude E, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature 2014;515:431–5.

19. Huang CH, Tsai MS, Hsu CY, et al. Post-cardiac arrest myocardial dysfunction is improved with cyclosporine treatment at onset of resuscitation

but not in the reperfusion phase. Resuscitation 2011;82 Suppl 2:S41-7.

20. Armstrong PW, Gershlick AH, Van de Werf F, Group SS. Fibrinolysis or primary PCI in myocardial infarction. N Engl J Med 2013;369:280-1, discussion 281.

21. Bartos JA, Matsuura TR, Sarraf M, et al. Bundled postconditioning therapies improve hemodynamics and neurologic recovery after 17 min of untreated cardiac arrest. Resuscitation 2015;87:7-13.

22. Shelat PB, Plant LD, Wang JC, Lee E, Marks JD. The membrane-active tri-block copolymer pluronic

F-68 profoundly rescues rat hippocampal neurons from oxygen-glucose deprivation-induced death through early inhibition of apoptosis. J Neurosci 2013;33:12287-99.

23. Wang T, Chen X, Wang Z, et al. Poloxamer-188 can attenuate blood-brain barrier damage to exert neuroprotective effect in mice intracerebral hemorrhage model. J Mol Neurosci 2015;55:240-50.

24. Emanuele M, Balasubramaniam B. Differential effects of commercial-grade and purified poloxamer 188 on renal function. Drugs R D 2014;14: 73-83.

25. Chiari PC, Bienengraeber MW, Pagel PS, Krolikowski JG, Kersten JR, Warltier DC. Isoflurane protects against myocardial infarction during early reperfusion by activation of phosphatidylinositol-3-kinase signal transduction: evidence for anesthetic-induced post-conditioning in rabbits. Anesthesiology 2005; 102:102-9.