Catheter-Based Transcoronary Myocardial Hypothermia Attenuates Arrhythmia and Myocardial Necrosis in Pigs With Acute Myocardial Infarction

Hiromasa Otake, MD, Junya Shite, MD, Oscar Luis Paredes, MD, Toshiro Shinke, MD, Ryohei Yoshikawa, MD, Yusuke Tanino, MD, Satoshi Watanabe, MD, Toru Ozawa, MD, Daisuke Matsumoto, MD, Daisuke Ogasawara, MD, Mitsuhiro Yokoyama, MD

Kobe, Japan

Objectives
This study evaluated the efficacy of catheter-based transcoronary myocardial hypothermia (CTMH) in pigs with acute myocardial ischemia.

Background
Although it has been suggested that hypothermia therapy can attenuate myocardial necrosis, few applications have been accepted for clinical use.

Methods
This study comprises 2 substudies. In both studies, pigs underwent 60 min of coronary occlusion and 180 min of reperfusion. In study 1, after 15 min of coronary occlusion with an over-the-wire-type balloon (OTWB), pigs in the hypothermia group (H) (n = 13) were directly infused with 4 °C saline into the coronary artery through the OTWB wire lumen (2.5 ml/min) for 60 min. Pigs in the normothermia group (N) (n = 15) received the same amount of 36.5 °C saline. In study 2, pigs in the hypothermia-reperfusion group (HR) (n = 5) were infused with 4 °C saline through the infusion catheter (8 ml/min) for 30 min with a later start (60 min after coronary occlusion), whereas simple reperfusion was used for the reperfusion group (R) (n = 6).

Results
Catheter-based transcoronary myocardial hypothermia was successful in both studies. In study 1, CTMH significantly decreased ventricular arrhythmia and the ratio of necrosis to ischemic risk area (H: 9 ± 2%; N: 36 ± 4%; p < 0.0001) with a significant reduction of enzyme leaks. In study 2, CTMH tended to reduce the ratio of necrosis (HR: 33 ± 2%; R: 45 ± 5%; p = 0.08). In both studies, CTMH significantly suppressed the increase of 8-iso-prostaglandin F\(_2\alpha\) while preserving the coronary flow reserve.

Conclusions
Catheter-based transcoronary myocardial hypothermia reduced myocardial necrosis while preserving coronary flow reserve, due, in part, to attenuation of oxidative stress. (J Am Coll Cardiol 2007;49:250–60) © 2007 by the American College of Cardiology Foundation

Coronary reperfusion therapy is widely performed in patients with acute myocardial infarction (MI), although its cardioprotective effect remains unsatisfactory. Reperfusion injury induces persistent myocardial necrosis in conjunction with increased oxidative stress (1–3) and activity of cytokines (4,5), which are believed to be major factors contributing to the deterioration of cardiac function after coronary reperfusion therapy. Although several agents, such as antioxidants (6), genes (7), and hormones (8), have been administered as adjuncts to coronary reperfusion, their efficacy for preventing ischemic damage has been found lacking. Findings from preliminary animal studies, however, have shown that mild hypothermia markedly ameliorates tissue damage after the onset of ischemia in many organs (9–12). As for the heart, several experimental studies have demonstrated that mild hypothermia can minimize myocardial necrosis resulting from acute MI (13–16). Ongoing research into systemic core cooling with an endovascular cooling system for patients with acute MI has shown its safety (17). With this method, however, sufficient cooling of the ischemic myocardium cannot be attained due to severe shivering caused by lowering the whole body temperature, so that the apparent myocardial protective effect is negated.

Therefore, we developed a new method involving cold saline infusion into an infarct-related coronary artery by means of a catheter. With this method, the cooling effect

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From the Division of Cardiovascular and Respiratory Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan. Manuscript received February 16, 2006; revised manuscript received June 9, 2006, accepted June 19, 2006.
was restricted to the ischemic myocardium, thus resulting in a substantial reduction in systemic complications. Furthermore, this technique is simple, so that it may be suitable for widespread clinical application. The purpose of the study presented here was to determine whether this method could effectively induce regional hypothermia as well as attenuate arrhythmia and myocardial injury in pigs with acute MI.

**Methods**

This study comprises 2 substudies. In study 1, we evaluated whether direct infusion of cold saline into the coronary artery could induce regional hypothermia and attenuate myocardial injury in pigs with ongoing ischemia. In study 2, to examine the clinical feasibility and efficacy of this procedure for acute MI, hypothermal-reperfusion therapy was initiated after a longer period of coronary occlusion and the result was compared with that obtained with simple immediate reperfusion-therapy in pigs with established MI.

**Subjects.** Thirty-nine Yorkshire pigs (28 for study 1, 11 for study 2) were used, and the study procedure conformed to the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

**Surgical preparation.** The pigs were sedated with intramuscular ketamine hydrochloride (20 mg/kg) and atropine sulfate (0.05 mg/kg). After tracheal intubation, deep anesthesia was induced with mechanical ventilation of oxygen and sevoflurane. Through a median sternotomy and systemic heparinization (100 U/kg intravenously/h), the pericardium was incised, and a deep body thermister (Coretemp CM-210, Terumo Co., Tokyo, Japan) to monitor the myocardial temperature at 5- to 6-mm depth was placed directly onto the area at risk of ischemia. A 6-F Swan-Ganz catheter (CCOM catheter; Terumo Co.) was advanced via the left internal jugular vein into the pulmonary artery to monitor cardiac output. A 5-F catheter was then inserted through the right internal jugular vein into the coronary sinus for blood sampling, while a 2-F micromanometer-tipped catheter (Millar Instruments, Houston, Texas) was advanced into the left ventricular (LV) cavity through a 5-F pigtail catheter via the right femoral artery for measuring peak positive first derivative of LV (LVdP/dt$_{max}$) and time constant of LV relaxation (tau). Finally, a 7-F angioplasty-guiding catheter (Heartrail, Terumo Co.) was used for entry into the left coronary from the right carotid artery.

**Experimental procedure.** Figure 1 provides an overview of the study protocols. First, baseline hemodynamics, myocardial and rectal temperature, and blood samples were obtained. After coronary angiography, an over-the-wire-type percutaneous transluminal coronary angioplasty balloon (OTWB) mounted on a 0.014-inch wire was advanced into the left anterior descending coronary artery (LAD), positioned at approximately one-third of the distance from the apex and inflated to occlude the LAD for 60 min. After 15 min of coronary occlusion, the pigs in study 1 were randomly assigned to the hypothermia or the normothermia group. For animals in the hypothermia group, cooled saline (4°C) was infused into the ischemic myocardium through the wire lumen of the OTWB at 2.5 ml/min (the maximum flow rate possible for this wire lumen). Pigs in the normothermia group were administered the same amount saline, but at 36.5°C in the same manner. After 60 min of coronary occlusion, reperfusion was achieved by complete deflation of the OTWB, and intracoronary saline infusion was continued for 15 min after reperfusion in both groups.

In study 2, the LAD was occluded at the same position by using an infusion balloon (Helios, Avantec, Vascular Corporation, Sunnyvale, California), which has a larger lumen than conventional OTWB so that a higher volume of saline could be infused. After 60 min of coronary occlusion, pigs were assigned to the hypothermia-reperfusion group or the reperfusion group. For pigs in the hypothermia-reperfusion group, cooled saline (4°C) was infused through the infusion balloon at 8 ml/min for 30 min followed by complete balloon deflation. For pigs in the reperfusion group, simple reperfusion was used after 60 min of coronary occlusion. In both studies, reperfusion was observed for 180 min.

**Incidence of arrhythmia.** Twenty-four-hour Holter recordings (Holtrec, Terumo Co.) were obtained and reviewed with the Holtrec Analysis System software to determine the total number of ventricular premature beats and sustained ventricular tachycardia (sVT). Ventricular premature beats were defined as the presence of at least 2 of 3 criteria: 1) atypical QRS configuration with alteration or inversion of the T wave; 2) post-extrasystolic pause; and 3) atrioventricular dissociation. Sustained ventricular tachycardia was defined as a fast ventricular rhythm of 15 or more beats in accordance with the Lambeth Conventions (18).

**Coronary flow velocity measurements.** Intracoronary Doppler flow measurements were performed with a 0.014-inch Doppler-tipped guidewire (FloWire; Volcano Therapeutics, Inc., Rancho Cordova, California) and a velocimeter (FloMap, Volcano Therapeutics Inc.) at baseline, 60 min, and 180 min after reperfusion. Doppler flow velocity spectra were analyzed on-line to determine time-averaged peak velocity (APV) during 2 cardiac cycles. After measurement of the baseline APV, the hyperemic APV for intracoronary papaverine (10 mg) injection was recorded, and the coronary flow reserve (CFR) was obtained as the ratio of hyperemic APV to baseline APV.
Blood sample analysis. Blood samples were obtained from coronary sinus at baseline and 180 min after reperfusion and stored at −80°C until analysis. To assess myocardial damage, creatinine kinase MB isozyme (CKMB) was measured with the aid of an Automated Chemiluminescence System (ADVIA Centaur; Bayer HealthCare, Leverkusen, Germany) with a detection limit of 5 ng/ml and the levels of cardiac troponin T (cTnT) were determined by means of a third-generation assay on an Elecsys 2010 (Roche Diagnostics, Indianapolis, Indiana), with a detection limit of 0.10 ng/ml. Production of 8-iso-prostaglandin F$_2$α (8-iso-PGF$_{2α}$) was quantified for assessment of the oxidative stress by using an 8-Isoprostane EIA KIT (Cayman Chemical Company, Ann Arbor, Michigan).

Measurement of myocardial area at risk and necrosis. At the end of the experiment, LAD was reoccluded, and 2% Evans blue was injected into the LV. After euthanasia, the heart was rapidly excised, and cut transversely into 0.5-cm thick slices, which were then photographed to identify the ischemic risk regions (not stained blue) and incubated in 2% triphenyl tetrazolium chloride for 20 min to delineate the area of necrosis. The ischemic and necrotic areas were traced and planimetered on each slice, and the results were summed to calculate the ratio of total necrotic area to ischemic area.

Histopathological assessment. Left ventricular sections from the ischemic area from both groups were collected at the end of study 2 and fixed with 10% formalin for ≥48 h, frozen in OCT compound, sliced into 5-μm sections, and stained with hematoxylin-eosin for light microscopic examinations.

Determination of myocardial water content. One aim of study 2 was to evaluate whether intracoronary saline infusion may cause myocardial edema. Post-experimental myocardium samples of 0.3 g were obtained from both the subendocardial and epicardial side of the ischemic area. The
tissue was weighed and desiccated for 48 h at 80°C, and the percentage of water content was calculated as (wet weight − dry weight)/(wet weight) × 100.

Statistical analysis. Statistical analysis was conducted with a commercially available software package (StatView 5.0, SAS Institute Inc., Cary, North Carolina). The differences between variables of the 2 groups were compared by using Student unpaired t test. Comparison of the ratio of pigs with sVT was performed with Fisher exact test. Two-way repeated measures analysis of variance followed by Bonferroni's multiple-comparison t test was used to compare serial measurements of hemodynamic and coronary flow parameters. Results were expressed as the mean ± SEM, and a p value <0.05 was considered significant.

Results

Study 1. AMELIORATIVE EFFECTS OF HYPOTHERMIA ON ONGOING MYOCARDIAL ISCHEMIA. One animal in the hypothermia and 3 in the normothermia group died of ventricular fibrillation, and their data were excluded from analysis. The body weight and LV weight of the remaining animals (hypothermia: 12; normothermia: 12) showed no significant differences between the 2 groups.

CHANGES IN MYOCARDIAL TEMPERATURE ASSOCIATED WITH REGIONAL HYPOTHERMIA. Figure 2A shows the changes in ischemic myocardial temperature. The baseline myocardial temperature of the 2 groups did not differ, but myocardial and rectal temperature of the normothermia group did not change throughout the study, whereas myocardial
temperature of the hypothermia group started to decrease immediately after the start of cold saline infusion and reached a minimum (33.1 ± 0.5°C; 3.2°C absolute reduction) just before reperfusion. The temperature then increased gradually after reperfusion. In contrast with the dramatic change in myocardial temperature, rectal temperature remained constant for the hypothermia group (data not shown).

**INCIDENCE OF ARRHYTHMIA.** The total number of ventricular premature beats was significantly less for the hypothermia group than for the normothermia group (246 ± 66 vs. 476 ± 30, p = 0.019), as was the ratio of pigs with at least 1 episode of sVT during the study (33% vs. 73%, p = 0.024 by Fisher exact test).

**HEMODYNAMICS AND LV FUNCTION.** Figure 3 shows the changes in hemodynamic parameters. Mean arterial pressure, heart rate, and cardiac output were similar for the 2 groups. Systolic function assessed by LVdP/dt max for the hypothermia group was markedly preserved at 60 min after reperfusion (103 ± 10.4% vs. 73.8 ± 6.7%, p < 0.001 by Bonferroni’s multiple-comparison t test). Diastolic performance assessed by tau did not show any statistical difference between the 2 groups.

**DOPPLER FLOW WIRE ASSESSMENT OF CORONARY BLOOD FLOW.** Baseline CFR of the 2 groups showed no difference; however, whereas the hypothermia group maintained its baseline level throughout the study, that of the normothermia group had decreased significantly 60 min and 180 min...
The temperature began to decrease after the start of cooling and reached a level similar to that of the hypothermia group in study 1 (33.1 ± 0.4°C; 3.4°C absolute reduction) even after complete deflation of the balloon. As for CFR levels, the hypothermia-reperfusion group showed a higher CFR than that for the reperfusion group 60 min after reperfusion (2.14 ± 0.12 vs. 1.49 ± 0.26; p = 0.0001 by Bonferroni’s multiple-comparison t test) (Fig. 4B). Blood sample analysis demonstrated that Δ8-iso-PGF₂α for the hypothermia-reperfusion group was significantly smaller than that for the reperfusion group (−1.20 ± 1.24 pg/ml vs. 3.50 ± 0.87 pg/ml; p = 0.023), whereas ΔCKMB and ΔcTnT showed no significant differences between the 2 groups (ΔCKMB: 40.7 ± 9.9 ng/ml vs. 46.7 ± 11.1 ng/ml, p = 0.71; ΔcTnT: 1.78 ± 0.64 ng/ml vs. 2.26 ± 0.27 ng/ml, p = 0.56) (Fig. 5B).

Although the ischemic risk area was the same for both groups (12 ± 1% vs. 13 ± 3%; p = 0.72), the necrotic area for the hypothermia-reperfusion group tended to be smaller than that for the reperfusion group but without statistical significance (33 ± 2% vs. 45 ± 5%, p = 0.080) (Fig. 6B). Myocardial water content showed no difference between the 2 groups, indicating that saline infusion did not cause myocardial edema (Fig. 9). Histologic examination with hematoxylin-eosin staining disclosed no significant differences in gross hemorrhage, microscopic hemorrhagic infarction, or inflammatory cell infiltration between the 2 groups.

Discussion

In this study, we achieved regional myocardial hypothermia by means of cold saline infusion via an OTWB...
catheter without accompanying hemodynamic deterioration or other adverse effects. Furthermore, hypothermia preserved CFR and dramatically reduced ongoing myocardial ischemia-related injury together with a reduction in ventricular arrhythmias and the extent of myocardial necrosis and attenuation of oxidative stress. Moreover, use of the infusion catheter, which makes it possible to infuse a larger amount of cold saline, enabled us to attain regional hypothermia without coronary artery occlusion. Indeed, once MI was established, hypothermia could not provide the same beneficial effects for ischemic necrosis as in the ongoing ischemia model. However, considering that this method suppressed the increase in 8-iso-PGF$_{2\alpha}$ and maintained the CFR level, regional hypothermia may have some cardioprotective effect even in the case of established MI.

**Figure 5** Enzyme Leaks and Oxidative Stress by Study

Comparisons of $\Delta$CKMB, $\Delta$cTnT, and $\Delta$8-iso-PGF$_{2\alpha}$ for the 2 groups in study 1 (A) and in study 2 (B). Data are expressed as mean ± SEM. CKMB = creatinine kinase MB isozyme; cTnT = cardiac troponin T; 8-iso-PGF$_{2\alpha}$ = 8-iso-prostaglandin F$_{2\alpha}$; $\Delta$ = the change in values between baseline and 3 h after reperfusion.
Previous studies of hypothermia for the prevention of ischemic injury. Hypothermia is currently an established method used not only for surgical procedures such as cardiopulmonary bypass surgery and organ transplantation, but also in several high-risk clinical settings, including acute ischemic stroke, traumatic brain injury, and cardiac arrest (19–21). Although hypothermia has gained much attention primarily for its neuroprotective effects (19–21), recent research has provided evidence of its efficacy for myocardial protection as well (13–16). Dave et al. (16) succeeded in reducing the extent of myocardial infarcts by 49% with cold saline perfusion of the pericardiac cavity. They also demonstrated that hypothermia by direct application of an ice-filled bag to the risk zone initiated after 10 min of occlusion reduced infarct size by 50% (14). Although these studies clearly demonstrate the effectiveness of hypothermia for MI, the methods used to achieve hypothermia are too invasive to be implemented in clinical settings. Whereas systemic hypothermia attained with an endovascular cooling device for human acute MI has been shown to be safe, cooling of the ischemic myocardium is too slow, so that this method has not yet proven itself to be effective (17). Additionally, many patients undergoing systemic hypothermia suffered from episodic shivering due to reduced core body temperature.

Advantages of catheter-based transcoronary myocardial hypothermia. This study demonstrated that cold saline infusion into the MI-related coronary artery successfully lowered myocardial temperature. One advantage of our method is that the localized effect within the ischemic myocardium is enhanced while systemic effects are reduced.
Moreover, this method entailed few complications such as hemodynamic instability, coronary vasoconstriction, and bradycardia. On the contrary, we found that hypothermia-treated pigs were electrically more stable than control pigs. Although the mechanisms of the antiarrhythmic effect of hypothermia remain uncertain, regional hypothermia may help suppress myocardial electrical irritability during ischemia and reperfusion.

In study 1, regional myocardial hypothermia effectively reduced the elevation of CKMB and cTnT levels after reperfusion. Histochemical studies showed that hypothermia led to a 75% reduction in infarct size compared with that for the normothermia group. In spite of a modest decline in myocardial temperature, this cardioprotective effect was relatively pronounced compared with previous reports for cooling from the epicardium. We suggest that the success of our method may be primarily attributable to the route used for cooling, because transcoronary hypothermia may lower myocardial temperature more homogeneously, and thus more effectively, than cooling from the epicardium.

Also, the pigs treated with hypothermia showed higher CFR in the infarct-related coronary artery than the normothermia-treated pigs after reperfusion. Because CFR can be used as an indirect parameter for evaluating the function of coronary circulation (22–24), preservation of CFR in the hypothermia-treated pigs reflects better coronary microcirculation. Dae et al. (15) used sestamibi autoradiography to demonstrate that hypothermia preserves microvasculature functions, whereas Hale et al. (25) showed that hypothermia significantly improves coronary reflow and reduces the no-reflow area in a rabbit MI model.
finding is thus in agreement with that of previous studies, namely, that hypothermia appears to improve coronary flow in MI.

One possible mechanism for the myocardial protection provided by hypothermia involves diminished metabolic demand on the ischemic myocardium. Previous animal studies have shown that hypothermia increases myocardial adenosine triphosphate preservation during both ischemia and reperfusion (26). From the fact that a significant reduction in infarct size was seen only in study 1, the reduction in metabolic demand within the ischemic myocardium may be one of the major mechanisms of the cardioprotection provided by hypothermia. Using an isolated rat liver model of ischemia and reperfusion, Zar et al. (27) showed that hypothermic perfusion reduced the formation of reactive oxygen species as well as post-ischemic vascular resistance compared with findings for normothermic-perfusion. It is further known that oxidative stress plays an important role in the deterioration of cardiac function (28), and that excessive stress induces tissue necrosis. Moreover, a previous study has shown that antioxidant vitamin C restores coronary microcirculatory function (29). The findings of only our study, therefore, do not clearly rule out the possibility that the reduction in 8-iso-PGF$_{2\alpha}$ may be a result rather than mechanisms. It was demonstrated, however, that isoprostanes themselves possess biological activities such as vasoconstriction (30), and free radicals are thought to mediate reperfusion injury. These facts may support the hypothesis that the cardioprotective effect of hypothermia is, in part, due to reduced oxidative stress.

**Study limitations.** In this study, the duration of coronary occlusion was 60 min, which is unrealistic for acute human MI. Because pigs tend to be much more frail than humans when it comes to ischemia, nearly all cells in the ischemic area in pigs hearts become necrosed after only 75 min of coronary occlusion (31). We, therefore, reduced the occlusion time to avoid death from heart failure and arrhythmia. In humans, however, the myocardial necrosis generally develops more slowly due to the greater collateral blood flow. Hypothermia may thus be beneficial for humans even when initiated later in the ischemic period. As for clinical application, a tool for monitoring the temperature, such as a thermo wire, would be helpful to keep the myocardial temperature within therapeutic levels.

On the basis of our preliminary findings, we believe that further experimental and clinical trials are warranted to determine whether adjunctive hypothermia therapy can limit infarct size during reperfusion therapy for MI.

**Conclusions.** We successfully achieved catheter-based regional hypothermia within the ischemic myocardium. This method preserved CFR and attenuated oxidative stress, which may be beneficial for the recovery of cardiac function in acute MI. We speculate that transcoryonary myocardial hypothermia may be an effective therapy, especially for patients with acute MI who are susceptible to ischemia-reperfusion injury.

**REFERENCES**


