primary techniques to induce myocardial ischemia in small rodents include the isolated perfused heart model and the selective coronary ligation model. However, both animal models have inherent advantages and disadvantages. Generally, isolated perfused hearts are used for acute experiments and are not suitable for assessing the chronic effects of myocardial ischemia. It is also difficult to extrapolate the results obtained on this system to intact animals, as isolated perfused hearts are no longer exposed to the internal milieu of circulation and metabolism. Constrictor devices to impede coronary artery blood flow have recently been used to induce ischemia in rabbit hearts, but these devices are too large for smaller rodents. With larger animals (eg, dogs, pigs), the chronic effects of ischemia can be investigated through the use of hydraulic occlusion devices that create transient myocardial ischemia in vivo, but, again, this method is not suitable for smaller animals. As a result, selective coronary ligation has been the primary method used to investigate long-term effects of transient or permanent myocardial ischemia in rats. Experiments using this method have been limited by variable ischemia-induced dysfunction and infarct size, as well as perioperative animal death.

The purpose of this study was to develop a new, less invasive rat model of transient myocardial ischemia in vivo.

Methods
Animals
Male Wistar rats (14–18 weeks old, 300–400 g body weight) were obtained from Hokudo (Sapporo, Japan). All procedures in this study were performed in accordance with the Institutional Animal Care and Use Committee of the Hokkaido University Graduate School of Medicine.

Devices
Guidewires (Choice PT-Plus 0.014-inch, Boston Scientific, Maple Grove, USA) were obtained from the cardiac catheterization laboratory of the Hokkaido University Hospital for re-use in this study. Guidewires were shortened to a length of 30 cm to improve their maneuverability. An infusion catheter (Target, Fremont, USA) was shortened to a length of 20 cm and used as a microcatheter to induce myocardial infarction.

Wire-Induced Transient Myocardial Ischemia
Rats were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg). A prophylactic dose of lidocaine (2 mg/kg, ip) and heparin (200 units/kg, sc) was injected to minimize the incidence of ischemia-induced ventricular arrhythmia and thromboembolism, respectively. Anesthetized rats were placed on a polystyrol board in a supine position, and a skin incision was made along the neck. The right common carotid artery was exposed and secured with ties at the distal end. A 24-G hypodermic needle (Vitaflon Plus, Becton Dickinson, USA) was introduced into the carotid artery under fluoroscopy. Transmural myocardial ischemia was confirmed by ST-segment elevation and by the appearance of left ventricular wall motion abnormalities on the echocardiogram. Reversibility of the wire-induced myocardial ischemia was demonstrated by complete resolution of both ST-segment elevation and wall motion abnormalities after removing the wire.
blood leaking from the puncture site. A 0.014-inch guidewire with J-shaped tip was advanced into the ascending aorta via the outer sheath under fluoroscopic guidance. In the case of the left coronary arteries, the wire tip was gently attached to the left coronary cusp of the aortic valve. The guidewire was advanced into the left coronary artery (LCA) by a counter clockwise rotation of the torque control device that was attached to the proximal end of the wire. In the case of the right coronary arteries, the guidewire was introduced by a clockwise rotation just above the right coronary cusp. Once the guidewire entered the coronary artery, the wire was slowly advanced until the electrocardiogram displayed ST-segment elevation. Although the body size of the rats used in this study was relatively large (300 to 400 g body weight, 1–2 year old), the inner diameter of ostial coronary arteries was nearly the same as the outer diameter of the guidewire (0.014 inch or 0.36 mm). Thus, a guidewire advanced into the coronary artery a few millimeters completely interrupted the blood flow to the downstream vascular bed, resulting in transmural myocardial ischemia. The guidewire was gently removed following 60 s of coronary obstruction. At the end of the experiment, the outer sheath was removed, and the skin incision was closed after the proximal carotid artery was securely tied. Transient myocardial ischemia was achieved in both the right and LCA (n=10 for each group). In order to assess reproducibility, some rats were exposed to wire-induced myocardial ischemia for 3 periods of 2 min each with a 2-min interval between each ischemic period.

Fig 1. Wire-induced myocardial ischemia in rats. A guidewire was advanced into (A) the left coronary artery (LCA) or (B) the right coronary artery (RCA). Electrocardiograms (control vs 45 s post-coronary occlusion) are also shown.
Electrocardiography and Echocardiography

Standard 12-lead electrocardiogram (ECG) was recorded throughout the experiment using a RM6000 multi-channel recorder (Nihon Kohden, Tokyo, Japan). Ten 27-G short needles were placed in the subcutaneous tissue and connected to each ECG lead. M-mode echocardiogram was recorded in 5 rats using the SSD-5500 ultrasound system equipped with a 10 MHz probe (Aloka, Tokyo, Japan). In order to obtain the echocardiographic indices, wire-induced myocardial ischemia was created for 2 min. On the M-mode echocardiogram performed at papillary muscle level, left ventricular end-diastolic dimension (LVDd) and end-systolic dimension (LVD) were measured, and fractional shortening (FS) was calculated. For videorecording the temporal change of ventricular wall motion and chamber size, 30 s of ischemia was created so as to minimize the movement of the ultrasound probe (Fig 3).

Irreversible Myocardial Ischemia Unduced by Microcatheter-Based Embolization

Once the wire was introduced into the rat’s coronary artery, an infusion catheter was advanced over the wire, and the wire was gently removed after the microcatheter tip was attached to the orifice of the coronary artery. Myocardial infarction was then induced by injecting a sepharose beads suspension (100 ml of 30% slurry, average diameter 90 mm) directly into the coronary artery through the infusion catheter.

Statistical Analysis

Data are reported as mean±SD. Data were analyzed by two-way repeated-measures ANOVA, and Bonferroni’s test was used to compare differences among multiple groups (StatView, Tokyo, Japan). Results were accepted as statistically significant when the p value was less than 0.05.

Results

Wire-Induced Myocardial Ischemia in Rats

After introducing the guidewire into the LCA, a significant elevation in ST-segment was observed in all chest leads (V1–V6), suggesting anterior left ventricular transmural wall ischemia (Fig 1A). Wire introduction into the right coronary artery (RCA) provoked ST-segment elevation in II, III, aVF, V1 and V2 (Fig 1B). These results confirmed that intraventricular septum in rats was perfused by RCA as reported previously.6 During the ischemic period, premature ventricular beats were observed occasionally. Ventricular tachycardia or fibrillation, however, were successfully suppressed by the prophylactic use of lidocaine.

Reversibility and Reproducibility of Wire-Induced Myocardial Ischemia

Myocardial ischemia was induced several times in the same coronary artery via repeated wire introductions without causing vascular injury. As illustrated in Fig 2, ST-segments of III and aVF were elevated when a guidewire was introduced into the RCA. Following 2 min of wire-induced coronary occlusion, the wire was removed from the coronary artery, and the ST-segments gradually returned to the baseline within 30 s. In most cases, however, it took nearly 1 min before complete recovery of ECG changes. When a guidewire was advanced into the RCA again, exactly the same ECG alterations were observed.

Echocardiographic Indices During Wire-Induced Myocardial Ischemia

Echocardiographic evaluation confirmed reversibility of
wire-induced myocardial ischemia. Under control conditions, LVDd, LVDs and FS were 5.3±0.2 mm, 2.9±0.2 mm and 46.7±2.5%, respectively. Anterior left ventricular wall dyskinesis was noted following wire introduction into the LCA. The measurements for LVDd and LVDs gradually increased during myocardial ischemia and reached a plateau by 30 s after coronary occlusion (LVDd, LVDs and FS after 45 s of coronary occlusion were 7.1±0.2 mm, 5.4±0.2 mm and 24.0±3.7%, respectively; \( p<0.01 \) vs control in all 3 echocardiographic indices). The guidewire was removed from the LCA after 2 min of occlusion, and the anterior left ventricular wall motion recovered over 20 s, which was faster than ECG recovery. Thirty seconds after the removal of the guidewire, LVDd, LVDs and FS were 5.9±0.1 mm, 3.3±0.1 mm and 44.1±2.8%, respectively. The exact same temporal changes in the left ventricular diameter during transient myocardial ischemia was reported in the patients treated with balloon angioplasty. M-mode echocardiogram was performed during the course of wire-induced ischemia (see Fig 3 for echocardiography in a representative animal). Echocardiographic evaluation was performed in 5 rats, and the results are summarized in Fig 4.

**Myocardial Infarction Induced by Microcatheter-Based Embolization**

Once the guidewire was successfully introduced into coronary artery, a microcatheter was advanced over the guidewire. Under fluoroscopic guidance, the microcatheter tip was carefully placed at the orifice of the coronary artery, and the guidewire was gently removed. Thrombotic agents were injected via the microcatheter to interrupt coronary blood flow and create myocardial infarction. In order to visualize the area perfused by each coronary artery, sepharose beads (average diameter 90 mm) were labeled with ethidium bromide and injected via the microcatheter. The infarct area was visualized under ultra violet illumination (Fig 5).
Novel Rat Model of Myocardial Ischemia

Discussion

Animal models that mimic human cardiovascular diseases are critical for the investigation of molecular pathology, pathophysiology, and potential therapeutic interventions. In general, small animals, such as rats and hamsters, are used because of their ease of handling and care, as well as low cost. In recent times, the most common used models of myocardial ischemia in rats include selective coronary ligation with thoracotomy and isolated perfused hearts (Langendorff preparation or working heart).

The isolated perfused rat heart model allows for control of perfusion pressure, coronary flow and oxygen supply and can accurately measure left ventricular pressure and wall tension. However, this ex vivo model is only applicable to acute states, and generalizations to in vivo states are limited. Chronic effects of myocardial ischemia have been investigated with the selective coronary ligation model, but the high perioperative mortality rate (15–60%) limits the usefulness of this method. Animal death with this method may result from acute congestive heart failure, lethal arrhythmia, or via complications from the procedures involved (thoracotomy, tracheal intubation and mechanical ventilation).

In the present study, we induced transient myocardial ischemia in the rats’ hearts using a hydrophilic-coated guidewire that was originally designed for the percutaneous transluminal coronary intervention procedures in patients with ischemic heart disease. Under fluoroscopic guidance, wires were selectively advanced into the coronary artery of the rats. Because the inner diameter of the proximal coronary arteries of the rats were smaller than the outer diameter of the guidewire (0.014 inch or 0.36 mm), blood flow to the downstream vascular bed was completely interrupted, resulting in transmural myocardial ischemia.

In preliminary experiments, an uncoated guidewire with a spring-coiled tip was used. However, this resulted in vascular injury with irreversible ST-segment elevation, likely due to coronary dissection and/or thrombotic acute coronary occlusion (data not shown). In order to reduce mechanical stress to the luminal surface of coronary arteries, guidewires with a hydrophilic coating were used. Present experiments demonstrated that even with repeated insertions of these coated guidewires, induced myocardial ischemia was fully reversible.

Based on the miniaturized catheterization technique, this new model of transient myocardial ischemia circumvents features. First, this procedure can create transient myocardial ischemia in small animals much less invasively than coronary ligation. In addition, the method created myocardial ischemia in the same coronary system repeatedly without vascular injury and resulted in uniform induction of myocardial ischemia (Fig 4). Finally, the wire-induced ischemia model can be modified to create irreversible myocardial ischemia (ie, myocardial infarction). As shown in Fig 5, results obtained by this catheter-based infarction model were comparable to those obtained with the coronary ligation model.

In the present study, we describe a novel approach to induce myocardial ischemia in rat hearts. Our data demonstrate that this model is a powerful and useful tool for the pharmacological, physiological, pathological and molecular study of ischemic heart disease.

References