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# Different Mechanisms of Ischemic Adaptation to Repeated Coronary Occlusion in Patients With and Without Recruitable Collateral Circulation

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***Objectives.*** **The aim of this study was to investigate the interaction between ischemic preconditioning (IP) and collateral recruitment (CR) during ischemic adaptation in patients.**

***Background.*** **The mechanism of ischemic adaptation still remains controversial in humans.**

***Methods.*** **The clinical, electrocardiographic, hemodynamic and echocardiographic responses to three 150-s occlusions of the left anterior descending coronary artery were assessed in relation to CR in 18 patients with effort angina undergoing elective percutaneous transluminal coronary angioplasty.**

***Results.*** **During the first occlusion, recruitable collateral circulation (RCC) to the occluded myocardium was detected by myocardial contrast echocardiography in 6 patients (Group C) and was not seen in 12 (Group N). In Group N, all patients manifested signs of severe ischemia during each inflation. However, their symptoms and ST segment shift significantly decreased from the first to the third occlusions, suggesting the occurrence of IP. The elevation of mean pulmonary artery pressure and deterioration of anterior wall motion were comparable between the first and the third occlusions in Group N. In contrast, myocardial ischemia was significantly less marked during occlusion in Group C than in Group N, and no preconditioning effect was observed. The extent of RCC did not differ between the first and the third occlusions in each group.**

***Conclusions.*** **Both IP and CR may play independent roles in ischemic adaptation in humans. With RCC, myocardial ischemia was greatly reduced. Without RCC, preconditioning clinically and electrocardiographically lessened myocardial ischemia but failed to preserve left ventricular function.**

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Transient coronary artery occlusion during percutaneous transluminal coronary angioplasty (PTCA) provides an excellent human model of myocardial ischemia, and thus it has been used for the investigation of ischemic preconditioning (IP) in patients (1–5). Clinical, electrocardiographic (ECG) and hemodynamic data have all revealed a decrease in the extent of myocardial ischemia after the initial inflation (1,2), supporting the occurrence of IP as proposed by experimental studies (6–10). Deutsch et al. (1) reported that the second coronary occlusion is characterized by less subjective anginal pain, less ST segment shift, a lower mean pulmonary artery pressure and less myocardial lactate production than with the first occlusion. In contrast, other investigators (3–5) found no evidence that myocardial protection against ischemia is induced by previous coronary occlusion. Dupouy et al. (3) recently reported that there was no difference in intracoronary ECGs, hemodynamic changes and echocardiographic changes during the second or third balloon inflations compared with the first. One possible explanation for the discrepancies between these preconditioning studies in patients may be the variety of study protocols and subjects as well as differences in the methods used to assess collateral flow and in the duration of coronary occlusion. In particular, the assessment of collateral flow seems likely to have been inadequate in previous studies because most used coronary angiography (CA) to visualize collateral circulation (1–5) and did not investigate recruitable collateral circulation (RCC) during coronary occlusion (1,3–5). CA only detects collateral vessels with a diameter >100 $\mu$m (11) and thus is not suitable for assessing the smaller collateral vessels in the myocardium that play an important role in protection against ischemia (12). Patients undergoing PTCA may have experienced frequent episodes of myocardial ischemia in daily life, and thus their collateral channels may open more easily than those of experimental animals (13–16). Accordingly, assessment of collateral recruitment (CR) during coronary occlusion is important in human preconditioning studies. However, there

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**Abbreviations and Acronyms**

CA = coronary angiography
CR = collateral recruitment
ECG = electrocardiogram, electrocardiographic
IP = ischemic preconditioning
MCE = myocardial contrast echocardiography (echocardiographic)
PTCA = percutaneous transluminal coronary angioplasty
RCC = recruitable collateral circulation

have been no human studies that quantitated RCC in the occluded microvascular bed in the myocardium at risk and investigated the interdependence between IP and CR during coronary occlusion.

Accordingly, we conducted the present study to investigate 1) how RCC develops in the occluded myocardium and influences myocardial ischemia during repeated coronary occlusion; and 2) whether IP is independent of CR in humans. For the assessment of collateral circulation, we used myocardial contrast echocardiography (MCE) in conjunction with PTCA (17). MCE, using the intracoronary injection of microbubbles of air, has been shown to be a promising method for investigating the distribution of myocardial perfusion (17–27) and is superior to CA for assessing collateral flow during coronary occlusion (17,22,25,26).

## Methods

The present investigation was approved by the ethics committee of the Osaka Police Hospital. Written informed consent was obtained from all patients.

**Patients.** The study included 18 patients (14 men, 4 women; 41 to 76 years old, mean age 61) with normal or minimally depressed normal left ventricular function who underwent elective PTCA of the left anterior descending coronary artery. Fourteen patients had single-vessel disease, and four had two-vessel disease. Their baseline characteristics are shown in Table 1. Patients with subtotal or total occlusion of the left anterior descending coronary artery were excluded from the study.

**Study protocol.** PTCA was performed through the femoral approach. An 8F left guiding catheter (Sherpa SL4.0, Medtronics Inc.) and a 5F right Judkins coronary arteriography catheter (Niporica JR 50 2H, Nippo Corp., Osaka, Japan) were advanced through sheaths placed in the femoral artery. A 7F pacing catheter (Lumelec, Cordis Corp.) was inserted into the pulmonary artery. After heart rate, aortic pressure, and pulmonary artery pressure were measured at baseline, right and left coronary arteriograms were obtained using a 10-ml injection of ioxaglate (Hexabrix-320, Tanabe Corporation, Tokyo, Japan) before balloon inflation. Then the severity of the coronary stenosis to be dilated was measured using digital

**Table 1.** Patient Characteristics

| Pt No. | Age (yr)/Gender | BP (mm Hg) | mBP (mm Hg) | PAP (mm Hg) | mPAP (mm Hg) | HR (beats/min) | WMS | Angio Collat Grade | Stenosis (%) | VD |
|---|---|---|---|---|---|---|---|---|---|---|
| Group N | | | | | | | | | | |
| 1 | 74/F | 118/72 | 98 | 24/10 | 17 | 79 | 0 | 1 | 72 | 2 (LAD, RCA) |
| 2 | 41/M | 126/76 | 94 | 20/10 | 15 | 70 | 0 | 0 | 57 | 1 (LAD) |
| 3 | 45/M | 130/90 | 110 | 16/7 | 11 | 83 | 1 | 0 | 50 | 1 (LAD) |
| 4 | 58/M | 120/80 | 100 | 23/12 | 17 | 68 | 0 | 0 | 53 | 1 (LAD) |
| 5 | 76/M | 114/52 | 72 | 20/8 | 15 | 52 | 1 | 0 | 62 | 1 (LAD) |
| 6 | 64/F | 112/70 | 90 | 26/13 | 22 | 88 | 1 | 1 | 49 | 1 (LAD) |
| 7 | 60/M | 82/68 | 75 | 22/13 | 17 | 94 | 0 | 0 | 69 | 2 (LAD, RCA) |
| 8 | 63/M | 160/82 | 114 | 18/8 | 13 | 107 | 0 | 2 | 76 | 1 (LAD) |
| 9 | 74/M | 130/74 | 94 | 28/14 | 19 | 72 | 0 | 0 | 79 | 1 (LAD) |
| 10 | 53/M | 126/76 | 96 | 30/16 | 23 | 69 | 0 | 0 | 72 | 1 (LAD) |
| 11 | 68/M | 138/64 | 92 | 19/8 | 13 | 78 | 0 | 0 | 61 | 2 (LAD, RCA) |
| 12 | 55/M | 150/94 | 128 | 27/9 | 16 | 76 | 0 | 0 | 51 | 1 (LAD) |
| Mean | 61 | 126/75 | 97 | 23/11 | 17 | 78 | | | 63 | |
| SD | 11 | 20/11 | 15 | 4/3 | 4 | 14 | | | 11 | |
| Group C | | | | | | | | | | |
| 13 | 61/M | 134/86 | 106 | 9/2 | 5 | 73 | 0 | 1 | 65 | 1 (LAD) |
| 14 | 56/M | 146/88 | 112 | 17/7 | 13 | 62 | 0 | 3 | 67 | 2 (LAD, LCx) |
| 15 | 66/M | 100/75 | 85 | 16/8 | 12 | 77 | 0 | 1 | 75 | 1 (LAD) |
| 16 | 65/F | 150/70 | 100 | 20/7 | 14 | 79 | 0 | 0 | 63 | 1 (LAD) |
| 17 | 55/M | 120/70 | 105 | 16/9 | 12 | 81 | 0 | 2 | 84 | 1 (LAD) |
| 18 | 62/F | 108/52 | 72 | 21/7 | 13 | 75 | 0 | 1 | 77 | 1 (LAD) |
| Mean | 61 | 126/74 | 97 | 17/7* | 12* | 75 | | | 72 | |
| SD | 5 | 20/13 | 15 | 4/2 | 3 | 7 | | | 8.2 | |

*$p < 0.05$ versus Group N. Angio Collat = angiographic collateral; BP = blood pressure; HR = heart rate; F = female; LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; M = male; mBP = mean blood pressure; mPAP = mean pulmonary artery pressure; PAP = pulmonary artery pressure; Pt = patient; RCA = right coronary artery; Stenosis = severity of the coronary stenosis; VD = vessel disease; WMS = wall motion score.

calipers, and angiographic coronary collateral vessel filling to the left anterior descending coronary artery was graded using a four-point scale (0 = none; 3 = good) (14). Baseline MCE was then performed using 2 ml of sonicated ioxaglate containing microbubbles of air (mean size 10 $\mu$m), which was separately injected into both the right and the left coronary arteries. Ioxaglate was sonicated for 20 s at 20 kHz and 60 W using a commercially available ultrasound homogenizer (Sonifier II, model 250, Branson Corp.), as previously reported (17,18,24). MCE images were acquired at or near the midpapillary muscle short-axis level using a commercially available phased array system (model SSH-160A ultrasound system, Toshiba Corp., Tokyo, Japan) with a 3.75-MHz transducer and recorded on a videotape recorder (model BR-6400, Victor Corp., Yokohama, Japan), from ~10 s before the injection of sonicated ioxaglate until contrast enhancement was no longer evident at a constant gain setting. After baseline CA and MCE were completed, the balloon catheter was advanced across the lesion to be dilated. Three 150-s balloon inflations were then performed at intervals of 3 min. At 135 s of the first and third inflations, MCE of the right coronary arterial injection of sonicated ioxaglate was performed to assess recruitable myocardial perfusion within the anterior segments. After these procedures, CA and MCE with injection of nonsonicated and sonicated ioxaglate into both the right and the left coronary arteries were performed. A 12-lead ECG was monitored during all procedures.

**Medications.** Long-acting nitrates, calcium channel antagonists or beta-adrenergic blocking agents were discontinued ≥24 h before the study. A continuous infusion of intravenous isosorbide dinitrate was started 1 h before the study at a constant rate (10 to 15 $\mu$g/min). Intravenous heparin (200 IU/kg) was administered before the first balloon inflation.

**Assessment of collateral blood flow.** The recorded MCE images were analyzed off-line with a Hewlett-Packard Sonos M2400A system. The video recordings were initially reviewed to select cycles in which the left ventricle was optimally visualized at the midpapillary short-axis level just before and after contrast injection, and the end-diastolic frame of the cycle showing the best delineation of the area with contrast enhancement was selected for analysis. The area supplied by collateral blood flow was determined by subtracting the area with contrast enhancement after the right coronary artery injection of sonicated ioxaglate at baseline or during balloon inflations from that after completion of all three balloon inflation procedures and then was expressed as a percentage of the total myocardial area at the short-axis level (19,20,25) (Fig. 1). Our intraobserver and interobserver variations for measuring the percent area supplied by collateral blood flow to the total myocardial area were 8.7 ± 4.4% and 9.1 ± 5.2%, respectively.

**Assessment of myocardial ischemia.** Anginal chest pain, ST-T segment shift in the precordial leads of the 12-lead ECG, hemodynamic changes and anterior wall motion were evaluated before and at 120 s of the first and third inflations. *Anginal pain* was assessed using a semiquantitative scale (0 = no anginal pain; 10 = severe anginal chest pain) (1). ST segment

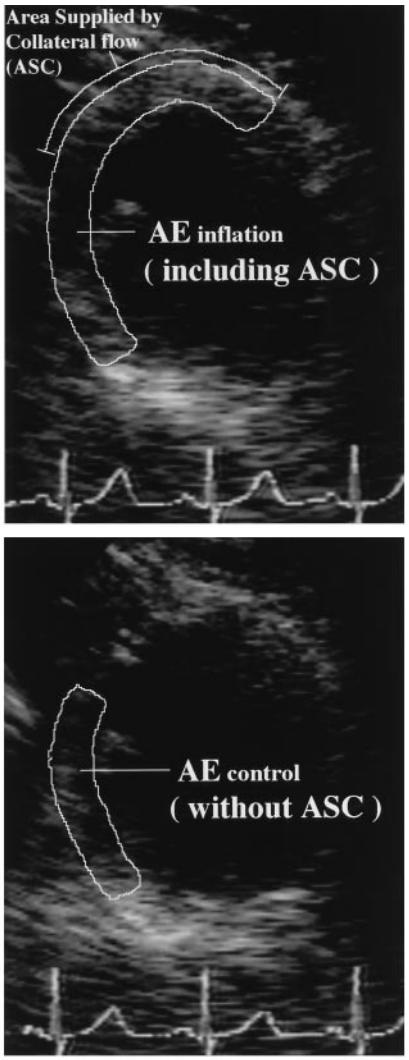


**Figure 1.** Two-dimensional echocardiographic short-axis view at the midpapillary level after injection of sonicated ioxaglate during balloon inflation (**top**) and after all angioplasty procedures (**bottom**). To quantitate the area supplied by collateral flow (ASC), the total myocardial area (TMA) and the area enhanced by contrast injection (AE) were digitized using a cursor controlled with a joystick. The area supplied by collateral flow was expressed as a percentage of the total myocardium using the following equation: $ASC = (AE_{inflation}/TMA_{inflation} - AE_{control}/TMA_{control}) \times 100$.

elevation was measured to the nearest 0.5 mm at 0.02 s after the J point, and the maximal value in the precordial leads was used for analysis. *Anterior wall motion abnormality* was graded according to the following scale: 0 = normokinesia; 1 = mild hypokinesia; 2 = hypokinesia; 3 = severe hypokinesia; 4 = akinesia or dyskinesia. ST segment elevation and wall motion abnormality were evaluated in blinded manner by two observers (F.I., A.S.) who viewed the ECGs or echocardiograms in random order and without knowledge of which patient was being presented. Differences in interpretation were resolved by consensus.

**Statistical analysis.** Results are expressed as mean value ± SD. Hemodynamic variables, collateral flow and indexes of

**Table 2.** Change in Area Supplied by Collateral Flow

| Pt No. | Baseline (%) | Inflation (%) 1st | Inflation (%) 3rd |
|---|---|---|---|
| Group N | | | |
| 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 |
| 4 | 0.22 | 0.12 | 0.11 |
| 5 | 0.31 | 0 | 0.30 |
| 6 | 0 | 0 | 0.30 |
| 7 | 0.37 | 0.39 | 0.50 |
| 8 | 0 | 0 | 1.16 |
| 9 | 0 | 1.50 | 1.40 |
| 10 | 1.40 | 1.10 | 1.50 |
| 11 | 0 | 0 | 2.84 |
| 12 | 0 | 2.60 | 3.00 |
| Mean | 0.2 | 0.5 | 0.9 |
| SD | 0.4 | 0.8 | 1.1 |
| Group C | | | |
| 13 | 1.82 | 13.26 | 10.74 |
| 14 | 4.68 | 12.94 | 13.97 |
| 15 | 0 | 12.41 | 17.90 |
| 16 | 0 | 20.03 | 17.93 |
| 17 | 2.88 | 18.12 | 18.87 |
| 18 | 12.17 | 14.37 | 23.43 |
| Mean | 3.6* | 15.2† | 17.1† |
| SD | 4.6 | 3.1 | 4.4 |

*$p < 0.05$, †$p < 0.001$ versus Group N. Pt = patient.

myocardial ischemia were compared between groups of patients with and without collateral flow and between the first and third inflations in each group using the unpaired and paired Student *t* test, Mann-Whitney *U* test and Wilcoxon single rank test. When serial changes in collateral flow and the indexes of myocardial ischemia were compared between the two groups, repeated measures analysis of variance was used, followed by the Scheffé F test. This analysis was performed on an Apple Macintosh computer using Statview IV software (Abacus Concepts). A p value $<0.05$ was accepted as indicating statistical significance.

## Results

**Clinical characteristics (Table 1).** During the first inflation, collateral blood flow was demonstrated in six patients (Group C) but not in 12 patients (Group N). There were no differences between groups with respect to age, gender, systemic blood pressure, mean blood pressure, heart rate and baseline wall motion score. There was a significant difference between the groups with respect to pulmonary artery pressure ($p < 0.05$). There was a moderate difference between the severity of the coronary stenosis to be dilated, although it was not statistically significant ($p = 0.08$).

**Table 3.** Anginal Score, ST Segment Shift and Wall Motion Score

| Pt No. | Anginal Score BL | Anginal Score Inf 1 | Anginal Score Inf 3 | ST Segment Shift (mV) BL | ST Segment Shift (mV) Inf 1 | ST Segment Shift (mV) Inf 3 | Wall Motion Score BL | Wall Motion Score Inf 1 | Wall Motion Score Inf 3 |
|---|---|---|---|---|---|---|---|---|---|
| Group N | | | | | | | | | |
| 1 | 0 | 6 | 3 | 0 | 0.3 | 0.2 | 0 | 4 | 4 |
| 2 | 0 | 10 | 6 | 0 | 0.6 | 0.3 | 0 | 4 | 4 |
| 3 | 0 | 3 | 3 | 0 | 0.8 | 0.8 | 1 | 4 | 4 |
| 4 | 0 | 10 | 6 | 0 | 0.3 | 0.2 | 0 | 4 | 4 |
| 5 | 0 | 1 | 2 | 0 | 0.5 | 0.2 | 1 | 4 | 4 |
| 6 | 0 | 3 | 1 | 0 | 0.15 | 0.05 | 1 | 4 | 4 |
| 7 | 0 | 10 | 10 | 0 | 0.2 | 0.1 | 0 | 4 | 4 |
| 8 | 0 | 6 | 3 | 0 | 0.1 | 0.15 | 0 | 4 | 4 |
| 9 | 0 | 0 | 0 | 0 | 0.2 | 0.15 | 0 | 4 | 4 |
| 10 | 0 | 10 | 5 | 0 | 0.5 | 0.4 | 0 | 4 | 4 |
| 11 | 0 | 0 | 0 | 0 | 0.6 | 0.2 | 0 | 4 | 4 |
| 12 | 0 | 10 | 8 | 0 | 0.55 | 0.5 | 0 | 4 | 4 |
| Mean | 0 | 5.7 | 3.9* | 0 | 0.40 | 0.27† | 0.25 | 4 | 4 |
| SD | 0 | 4.3 | 3.1 | 0 | 0.22 | 0.21 | 0.45 | 0 | 0 |
| Group C | | | | | | | | | |
| 13 | 0 | 0 | 0 | 0 | 0.1 | 0.1 | 0 | 1 | 2 |
| 14 | 0 | 0 | 0 | 0 | 0.05 | 0.05 | 0 | 1 | 1 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 18 | 0 | 4 | 0 | 0 | 0.1 | 0 | 0 | 4 | 1 |
| Mean | 0 | 0.67‡ | 0§ | 0 | 0.04§ | 0.03‡ | 0 | 1.3§ | 1§ |
| SD | 0 | 1.63 | 0 | 0 | 0.05 | 0.04 | 0 | 1.4 | 0.63 |

*$p < 0.05$, †$p < 0.01$ versus inflation 1. ‡$p < 0.05$, §$p < 0.01$ versus Group N. BL = baseline; Inf = inflation; Pt = patient.

**Table 4.** Hemodynamic Changes During Repeated Coronary Occlusion

| | mBP (mm Hg) | | HR (beats/min) | | mPAP (mm Hg) | |
|---|---|---|---|---|---|---|
| | BL | Occl | BL | Occl | BL | Occl |
| Group N | | | | | | |
| Inf 1 | 94.4 ± 17.2 | 90.0 ± 15.6 | 73.1 ± 12.8 | 73.7 ± 13.7 | 16.4 ± 3.4* | 23.7 ± 6.5†‡ |
| Inf 3 | 91.1 ± 14.5 | 87.4 ± 12.1 | 71.7 ± 15.0 | 71.1 ± 15.3 | 19.2 ± 6.1‡ | 24.0 ± 5.4‡‖ |
| Group C | | | | | | |
| Inf 1 | 96.7 ± 15.1 | 94.7 ± 13.1 | 74.5 ± 6.7 | 79.1 ± 4.3 | 11.5 ± 3.3 | 12.0 ± 3.3 |
| Inf 3 | 93.5 ± 14.2 | 95.0 ± 12.3 | 78.8 ± 4.4 | 80.3 ± 4.5 | 10.0 ± 2.6 | 11.7 ± 2.8 |

*$p < 0.05$, ‡$p < 0.01$, §$p < 0.001$ versus Group C. †$p < 0.05$, ‖$p < 0.01$ versus baseline. Data presented are mean value ± SD. Occl = during inflation; other abbreviations as in Tables 1 and 3.

**Collateral blood flow during repeated coronary occlusion.** In Group N, the area supplied by collateral flow at baseline and at the first and third inflations was, respectively, 0.2 ± 0.4%, 0.5 ± 0.8% and 0.9 ± 1.1% of the left ventricle. In Group C, the area supplied by collateral flow at baseline and at the first and third inflations was, respectively, 3.6 ± 4.6%, 15.2 ± 3.1% and 17.1 ± 4.4% of the left ventricle. There was a significant difference in the serial change of collateral flow between Groups N and C ($p < 0.0001$). There was a significant difference between Groups N and C in the area supplied by collateral flow at baseline ($p < 0.05$), the first inflation ($p < 0.001$) and the third inflation ($p < 0.001$) (Table 2). The area supplied by collateral flow did not differ between the first and third inflations in both Groups N and C.

**Symptoms during repeated coronary occlusion.** The serial change in anginal symptoms was significantly different between Groups N and C ($p < 0.01$). Before each inflation, all patients had no symptoms. In Group N, 10 of 12 patients developed anginal symptoms during the first inflation. The symptom score decreased in seven patients, increased in one and did not change in two at the third inflation. Overall, the third inflation was characterized by less severe anginal symptoms than the first inflation in Group N ($p < 0.05$). In contrast, only one patient in Group C (Patient 18) had anginal symptoms (anginal pain scale: 4) during the first inflation, which disappeared by the third inflation. There were significant differences between Groups N and C in the anginal symptoms noted during the first and third inflations ($p < 0.05$ and $p < 0.01$, respectively) (Table 3).

**ECG changes during repeated coronary occlusion.** There was a significant difference in the serial changes of ST-T wave shifts between Groups N and C ($p < 0.01$). Before each inflation, there was no ST-T segment shift in the precordial ECG leads in any patient. In Group N, all patients showed significant ST-T segment shift (0.40 ± 0.22 and 0.27 ± 0.21 mV at the first and third inflations, respectively). The magnitude of the ST-T segment shift was significantly less during the first inflation than during the third inflation in Group N ($p < 0.01$). In contrast, all patients in Group C showed only slight ECG changes. The ST-T segment shift was significantly smaller in Group C than in Group N during the first and third inflations ($p < 0.01$ and $p < 0.05$, respectively) (Table 3).

**Echocardiographic changes during repeated coronary occlusion.** Changes in the anterior wall motion score at baseline and during the first and third inflations are shown in Table 3. Serial changes in the anterior wall motion score differed significantly between Groups N and C ($p < 0.0001$). At baseline, normokinesia (grade 0) was seen in nine Group N patients and in all Group C patients. Three patients from Group N showed slight deterioration of wall motion (grade 1). During each inflation, anterior wall motion severely deteriorated and manifested akinesia (grade 4) in all Group N patients. In contrast, no akinesia was observed in Group C, except in Patient 15 during the first inflation. There were significant differences in wall motion score between Groups N and C during the first and third inflations ($p < 0.001$ and $p < 0.001$, respectively).

**Hemodynamic variables during repeated coronary occlusion.** Table 4 shows the hemodynamic responses to repeated coronary occlusions in Groups N and C. In both groups, mean blood pressure and heart rate did not change significantly with repeated coronary occlusion. Mean pulmonary artery pressure increased significantly during the first and third coronary occlusions in Group N ($p < 0.05$ and $p < 0.01$, respectively), whereas there was no change in Group C. Between the first and third inflations, there were no significant changes in mean pulmonary artery pressure at baseline and during inflation in each group, but there was a significant difference between Groups N and C in mean pressure during the first and third inflations ($p < 0.01$ and $p < 0.001$, respectively) as well as at baseline, before the first and third inflations ($p < 0.05$ and $p < 0.01$, respectively).

## Discussion

**Different responses to repeated coronary occlusion in patients with and without RCC.** Since Murry et al. (6,7) reported that an antecedent ischemic episode delayed irreversible cellular injury after coronary occlusion, several studies have been conducted, and the concept of IP has been established by experimental data excluding animals with high collateral flow. However, to our knowledge, IP has never been investigated independently of CR in humans because of the difficulty in assessing collateral blood flow in the occluded microvascular

bed. Because previous preconditioning studies (1–5) enrolled patients both with and without RCC or did not completely exclude CR, they could not investigate acquisition of ischemic tolerance in relation to CR. However, in the present study we assessed RCC in the myocardium at risk after coronary occlusion, using MCE in conjunction with PTCA, and could distinguish between patients with and without RCC. Our results showed that the response to repeated ischemic episodes was different in patients with and without RCC. In patients with RCC that was not enhanced by repeated coronary occlusion, myocardial ischemia was far less severe during coronary occlusion. In contrast, all patients without RCC had severe ischemia during each balloon inflation, but the third occlusion was characterized by less clinical and ECG evidence of myocardial ischemia than during the first occlusion, suggesting the acquisition of ischemic tolerance during repeated coronary occlusion. This observation in patients without RCC seems to be the first evidence that ischemic tolerance can be acquired independently of CR in humans because previous studies that showed a clinical preconditioning effect could not completely exclude the involvement of CR (1) or otherwise concluded that the acquisition of ischemic tolerance in the clinical setting was attributable to enhanced collateral circulation (2).

**Acquisition of myocardial ischemic tolerance independent of CR.** In the present study, acquisition of ischemic tolerance in humans was demonstrated by excluding the data from patients with recruitable collateral flow. Anginal symptoms and ST-T segment shift were less marked at the third inflation than the first in patients without RCC (Group N), supporting the concept of IP in humans, as has been shown in animal experiments (6–10). However, in addition to the lesser clinical and ECG evidence of myocardial ischemia, we also showed that previous coronary occlusions had no effect on preserving left ventricular function at the next occlusion. The extent of the elevation in mean pulmonary artery pressure and deterioration in anterior wall motion during the third inflation were comparable to those during the first inflation in Group N, whereas heart rate and mean aortic pressure did not differ both before and during each inflation. Because clinical symptoms and ECG changes generally appear in the later stage of myocardial ischemia after coronary occlusion compared with hemodynamic changes and regional wall motion abnormality (28), repeated coronary occlusion might have preconditioned the myocardium to raise the threshold for provoking myocardial ischemia.

Deutsch et al. (1) also suggested that repeated coronary occlusion induced IP, probably in the absence of collateral enhancement in the clinical setting. They concluded that no increase in collateral blood flow was induced by repeated coronary occlusion because coronary venous blood flow decreased during the second occlusion. Their data are consistent with ours but do not rule out the possibility that RCC appeared during the second occlusion in some of their patients.

Dupouy et al. (3) investigated IP in patients without severe coronary stenosis in whom RCC was considered to be insignificant. They concluded that brief episodes of ischemia did not induce IP, as evidenced by changes in intracoronary ECG and echocardiographic and hemodynamic variables in patients with no angiographic evidence of collateral circulation before coronary occlusion. Thus, their conclusions seemed different from ours. However, the difference between their findings and ours may be explained by the power of their study to find differences because of the limited number of patients. In fact, they found a nonsignificant but moderate decrease in ST segment elevation from the first to the third inflation (from $1.8 \pm 1.2$ to $1.35 \pm 0.9$ mV). Additionally, the duration of coronary occlusion was slightly longer in our study than in their study (150 vs. 120 s), which is another possible explanation. The present study seemed to shed further light on the question of whether IP occurs in patients and, if so, under what conditions. We clearly determined the presence or absence of RCC during each coronary occlusion in individual subjects, which, to our knowledge, has not been assessed in previous studies.

**Recruitment of collateral flow and myocardial ischemia.** Another goal of the present study was to investigate whether an increase in RCC in the occluded myocardium was induced by repeated coronary occlusion and how it influenced myocardial ischemia. MCE demonstrated RCC in six of our patients (Group C). The percent area supplied by recruitable collateral flow did not differ between the first and third inflations, so the presence or absence of CR seemed to be determined before the first coronary occlusion. In Group C, coronary stenosis was more severe, and the area supplied by collateral flow at baseline was larger than that in Group N, suggesting that the presence of "preexisting collateral channels" that are ready to open might be related to the immediate appearance of RCC. Cribier et al. (2) also described the striking development of collateral circulation during the first coronary occlusion compared with baseline CA, which agrees with our findings. However, unlike in their study, we did not observe enhancement of RCC with repeated coronary occlusion. This discrepancy may be explained by the different methods used for assessment of collateral circulation. Cribier et al. used CA to assess collateral circulation, which reflects epicardial collateral vessel filling but does not necessarily reflect myocardial perfusion in the occluded bed (18,22,25,26), especially immediately after coronary occlusion (17).

The magnitude of myocardial ischemia during each inflation in patients with RCC was significantly lower than that in patients without RCC. Except during the first inflation in Patient 15 (Table 1), no significant myocardial ischemia was seen with each inflation in patients with RCC. RCC eliminated the clinical, ECG, hemodynamic and echocardiographic evidence of myocardial ischemia from the time of the initial inflation. This observation in patients with RCC was in marked contrast to the preconditioning effect in patients without RCC, which only lessened the clinical and ECG features of myocardial ischemia after the initial inflation but not the hemodynamic and echocardiographic variables. Thus, the mechanisms underlying ischemic adaptation during repeated coronary occlusion appear to be different between IP and CR. The

mechanism of ischemic adaptation by CR is probably due to restoration of a sufficient oxygen supply to the ischemic myocardium to meet oxygen demand, whereas that due to IP is probably not and may be due to improved ischemic tolerance of cardiac myocytes (6–10).

**Mechanism of IP.** Our findings do not provide enough evidence to elucidate the mechanism of IP. Murry et al. (6) suggested that preconditioning by intermittent reperfusion due to washout of the catabolites that accumulate during ischemia might play a role in decreasing the extent of ischemia with subsequent balloon inflations. Other mechanisms, such as endogenous adenosine, have been reported in previous studies (8–10). Because adenosine may contribute to the recruitment of collateral circulation as well as making the myocardium more resistant to ischemic insults, endogenous adenosine may have contributed to the cardioprotective effect seen in the present study. We previously reported (29) that myocardial oxygen consumption was reduced in the second supine ergometric exercise test performed in patients with effort angina compared with that during the first exercise test. In that study, adenosine release was greater during the second exercise session than during the first. Although supply-type and demand-type ischemia may be different, reduced myocardial oxygen demand and enhanced adenosine release might have ameliorated myocardial ischemia during the subsequent balloon inflation in the present study.

The baseline differences between the two groups may at least partially explain the mechanism of ischemic adaptation. That the group with RCC had both moderately worse baseline coronary stenosis of the dilated vessel and significantly less elevated baseline pulmonary artery pressures, coupled with the observation that this group had significantly less marked myocardial ischemia during coronary occlusions, might mean that the group with RCC had already undergone IP through repeated episodes of angina, and, therefore, no additional IP occurs during coronary occlusions performed in the present study.

**Limitations of the study.** The present study had the following limitations: 1) For ethical reasons, intravenous administration of nitroglycerin was continued throughout the study in all patients. Nitroglycerin is reported to affect collateral circulation in dogs (30). However, the infusion was maintained at a constant rate throughout the study and thus probably had little influence on the results. 2) We only assessed collateral perfusion through the right coronary artery and did not assess recruitable collateral flow from the left coronary artery. However, it seems unlikely that assessment of left coronary artery collateral flow would greatly affect the results because a) it is remarkable how the two groups of patients are differentiated, even without the data of left to left collateral flow; and b) there was little discrepancy between the cardioprotective effect during coronary occlusion and the presence of collateral perfusion through the right coronary artery in the previous report (17).

**Clinical implications.** Although great attention has been focused on the phenomenon of IP, few data are available in the clinical setting. We first showed that IP is independent of CR in humans, which has previously been shown only in animal studies. These findings may help in understanding the discrepancies between clinical and experimental data as well as the mechanism of ischemic adaptation in patients with coronary artery disease.

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