Table 1. Comparisons of the Baseline Characteristics by Gender

	Women	Men	P value
Age (years; mean±SD)	70.6±0.2	65.8±0.1	<0.001
Age≥75 (%)	37.0	19.1	<0.001
BMI≥25 (%)	30.0	31.0	0.33
Prior MI (%)	20.9	27.8	<0.001
History of heart failure (%)	20.5	15.5	<0.001
Prior stroke (%)	15.6	17.1	0.068
Peripheral vascular disease (%)	4.0	8.8	<0.001
Atrial fibrillation (%)	6.1	7.1	0.078
Diabetes (%)	42.0	37.6	<0.001
Hypertension (%)	75.3	66.6	<0.001
Dyslipidemia			
LDL-C≥130 mg/dl (%)	43.4	35.0	<0.001
Triglyceride≥150 mg/dl (%)	33.2	36.1	0.016
HDL-C<40 mg/dl (%)	17.8	32.2	<0.001
Current smoker (%)	10.5	35.7	<0.001
CKD (eGFR<60 ml·min^{-1}·1.73-m^{-2}) (%)	52.3	35.7	<0.001
Anemia (hemoglobin<12 g/dl) (%)	44.3	18.4	<0.001
Multivessel disease (%)	65.0	63.5	0.16
Unstable angina (%)	8.0	7.4	0.37

BMI, body mass index; MI, myocardial infarction; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

significant differences in the prevalence of CAD between Caucasian and Asian patients, there are limited large-scale data for gender differences in the risk factor profiles and cardiovascular outcomes for Asian patients with CAD.

The purpose of the present study was to identify gender-based differences in risk factors and outcomes in Japanese patients undergoing coronary revascularization.

Methods

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the a priori approval by the Human Research Committees of all participating institutions. Because the study subjects were retrospectively enrolled, written informed consent was not obtained in concordance with the guidelines for epidemiological studies issued by the Ministry of Health, Labour and Welfare of Japan. However, 73 patients were subsequently excluded because of their refusal to participate in the study when contacted for follow-up.

Study Subjects

Participants of the Coronary REvascularization Demonstrating Outcome study in Kyoto (CREDO–Kyoto) registry have been previously described.[15,16] A total of 9,877 consecutive patients who underwent their first PCI or CABG between 2000 and 2002 from 30 institutions (**Appendix**) were registered.[15] The subjects evaluated in the present study were 9,688 patients comprising 2,845 women and 6,843 men after excluding patients with malignant diseases. The baseline characteristics, treatments, in-hospital outcomes, and cardiovascular events during follow-up, including death, myocardial infarction, stroke, and any repeated revascularization procedures, were compared between women and men.

Data Collection, Definitions and Follow-up

Clinical and analytical data for the study subjects were collected from hospital charts or databases in each center by independent clinical research coordinators (**Appendix**).[15] The baseline data for the patients included: age; gender; smoking habit; body mass index (BMI); mode of revascularization; and comorbidities such as hypertension, diabetes mellitus (DM), dyslipidemia (low-density lipoprotein cholesterol [LDL-C] ≥130 mg/dl, triglyceride ≥150 mg/dl, high-density lipoprotein cholesterol [HDL-C] <40 mg/dl), chronic kidney disease (CKD), anemia, peripheral vascular disease, history of heart failure, prior myocardial infarction, and prior cerebrovascular accident. DM was diagnosed by each physician based on the diagnosis and classification of DM of the expert committee.[17] CKD was defined as a glomerular filtration rate estimated by the Cockcroft–Gault formula of <60 ml·min^{-1}·1.73 m^{-2}. Peripheral vascular disease was defined as being present when the patients were being treated for carotid, aortic, and/or other peripheral vascular diseases or scheduled for interventions. Anemia was defined as a blood hemoglobin level of <12 g/dl. The patients were followed up with respect to mortality for a median of 3.6 years. All deaths were confirmed by medical records or telephone interviews with the patients' families, and death was regarded as being cardiovascular in origin unless obvious non-cardiovascular causes were identified. Myocardial infarction was defined according to the Arterial Revascularization Therapy Study.[18] In the present study, major adverse cardiovascular events (MACE) were defined as the composite of cardiovascular death, myocardial infarction, and stroke. Any revascularization procedures during follow-up included any PCI or CABG. Follow-up coronary angiography (CAG) was not performed routinely, but left to the discretion of the attending physician.

Statistical Analysis

All continuous variables were expressed as mean±SD. Differences in the baseline clinical characteristics and treatments between women and men were evaluated by the Pearson χ^2 test for categorical data and the Student's t-test for continuous

Table 2. Revascularization Procedures and Medications at Hospital Discharge

Treatment	Women	Men	P value
Revascularization procedures			
PCI (%)	70.4	69.2	0.21
Stent use (%)	81.4	82.1	0.45
No. implanted stents, mean±SD	1.23±0.02	1.23±0.01	0.75
CABG (%)	29.6	30.8	0.21
No. total grafts, No.±SD	2.40±0.02	2.41±0.01	0.79
Use of LITA (%)	85.7	90.4	<0.001
Use of RITA (%)	25.4	29.8	0.014
Off-pump procedures (%)	39.3	39.1	0.93
Medication at discharge			
ACEI/ARB (%)	35.0	31.8	0.002
β-adrenergic blockers (%)	16.6	16.6	0.96
Statins (%)	35.5	25.0	<0.001
Anti-platelet drugs (%)	94.9	95.5	0.15
Calcium channel blockers (%)	61.3	57.9	0.002
Nitrates (%)	63.0	61.8	0.25
Follow-up CAG			
All patients (%)	60.4	61.2	0.45
PCI group (%)	79.5	81.8	0.027
CABG group (%)	14.7	14.9	0.92

PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; LITA, left internal thoracic artery; RITA, right internal thoracic artery; ACEI, angiotensin-converting enzyme inhibiters; ARB, angiotensin II receptor blockers; CAG, coronary angiography.

data. To identify significant and independent prognostic factors for clinical outcomes, we listed the following potential baseline variables: age; gender; BMI≥25 kg/m^2; DM; prior myocardial infarction; history of heart failure; prior stroke; peripheral vascular disease; atrial fibrillation; hypertension; CKD; anemia; LDL-C≥130 mg/dl; triglyceride ≥150 mg/dl; HDL-C <40 mg/dl; current smoking; multivessel coronary disease; and unstable angina. The following cardiovascular pharmacotherapies at hospital discharge were also listed: statins; angiotensin-converting enzyme inhibitors (ACEI)/ angiotensin II receptor blockers (ARB); β-adrenergic blockers; anti-platelet drugs; calcium channel blockers; and nitrates. Survival analyses were performed using the Kaplan–Meier method and differences in survival between women and men were examined using a log-rank test. The Cox proportional hazards model was used to adjust the results for baseline differences between women and men. The model included baseline variables, medications at discharge and revascularization strategy (PCI/CABG). In the analysis for predictors of any coronary revascularization, a variable indicating whether follow-up CAG was performed was included in the model, because several previous studies reported that angiographic follow-up after stent implantations influenced the rates of repeat revascularization procedures.[19,20] Analysis for the predictors of repeat revascularization was also performed in the PCI-treated patients, because the incidence of repeat revascularization was distinctively higher in PCI-treated than CABG-treated patients and routine follow-up CAG is generally scheduled after PCI but rarely after CABG.

All analyses were conducted using the software JMP version 5 (SAS Institute Inc, Cary, NC, USA). All reported P-values were 2-sided. Values of P<0.05 were considered to indicate statistical significance.

Results

Baseline Characteristics

The baseline clinical characteristics of the women and men are listed in **Table 1**. The women were approximately 5 years older than the men, and 37% of the female patients were ≥75 years. Compared with men, women more frequently had many of the known cardiovascular risk factors such as history of heart failure, DM, hypertension, hyper-LDL-cholesterolemia, CKD, and anemia, while there were higher prevalences of prior myocardial infarction, peripheral vascular disease, hypo-HDL-cholesterolemia, and current smokers among the men. There were no significant differences in the rates of multivessel disease and unstable angina between the women and men.

Treatments

Comparisons of the treatments between women and men are shown in **Table 2**. Among the 9,688 patients, PCI was performed in 6,740 (69.7%) patients while CABG was performed in 2,948 (30.3%) patients. There were no significant differences in the selection of revascularization strategy (PCI or CABG) between the women and men. In the PCI-treated patients, the use of stents was comparable between women and men (81.4% vs. 82.1%), and the numbers of implanted stents per patient were also comparable between women and men (1.23±0.02 vs. 1.23±0.01). All stents used in the patients registered in the CREDO–Kyoto registry in 2000–2002 were bare metal stents. In the CABG-treated patients, the total numbers of grafts were similar between the women and men (2.40±0.02 vs. 2.41±0.01). However, use of the left internal thoracic artery (ITA) was less frequent in women than in men (85.7% vs. 90.4%, P<0.001).

With regard to medications at discharge, there were no significant differences between the women and men in the

Table 3. Crude In-Hospital Event Rates in Women vs. Men

	Women	Men	P value
In-hospital death (%)	1.4	1.2	0.45
MI (%)	3.8	2.6	0.002
Q-wave MI (%)	0.95	0.94	0.95
Non-Q-wave MI (%)	2.9	1.7	<0.001
Stroke (%)	0.70	0.75	0.82
Emergent PCI (%)	1.1	0.94	0.40
Emergent CABG (%)	0.21	0.19	0.45
Acute/subacute coronary occlusion (PCI group) (%)	1.0	0.68	0.17

Abbreviations see in Tables 1,2.

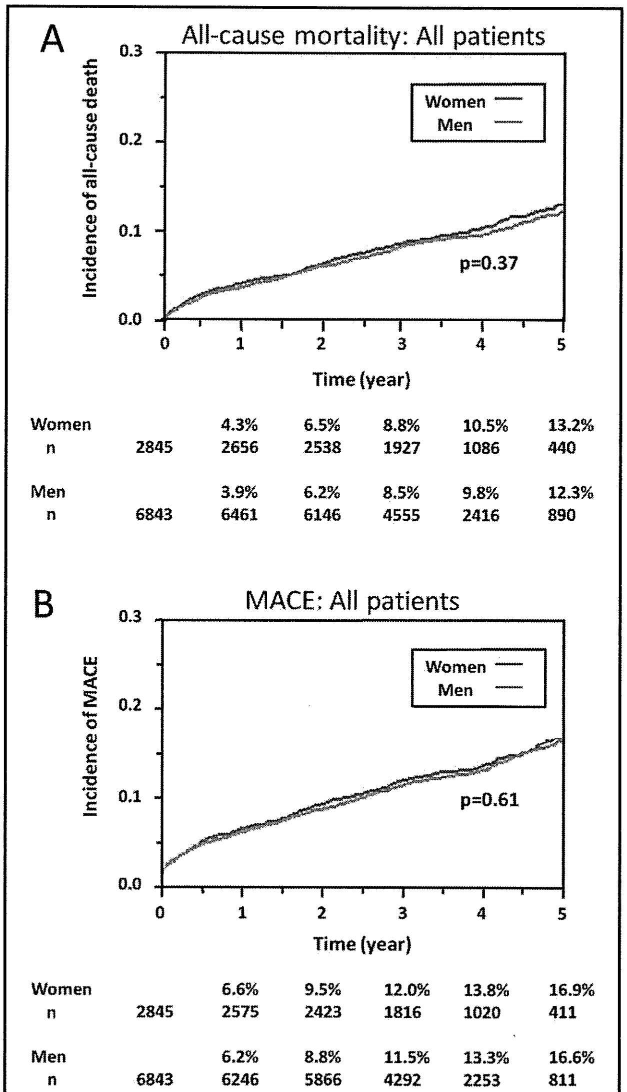

Figure 1. Incidence of all-cause death and major adverse cardiovascular events (MACE) in all patients. No significant differences were found between the women (red lines) and men (green lines) in the incidence of death or MACE. (**A**) Incidence of all-cause death in women vs. men. (**B**) Incidence of MACE in women vs. men.

proportions of patients treated with β-adrenergic blockers (16.6% vs. 16.6%), anti-platelet drugs (94.9% vs. 95.5%), and nitrates (63.0% vs. 61.8%). However, ACEI/ARB (35.0% vs. 31.8%, P=0.002), statins (35.5% vs. 25.0%, P<0.001), and calcium channel blockers (61.3% vs. 57.9%, P=0.002) were more frequently prescribed in women than in men.

The rate of follow-up CAG was comparable between women and men (60.4% vs. 61.2%) in all subjects. However, when the PCI-treated patients were analyzed, the rate of follow-up CAG was significantly lower in women than in men (79.5% vs. 81.8%, P=0.027).

In-Hospital Outcomes

There were no significant differences in the incidences of in-hospital death, Q-wave myocardial infarction, and stroke between women and men. However, the incidence of non Q-wave MI (2.9% vs. 1.7%, P<0.001) was significantly higher in women than in men (**Table 3**).

Mortality and MACE During Follow-up

Figure 1 indicates the unadjusted incidences of all-cause death and MACE during follow-up. No significant differences were shown between women and men in the incidences of all-cause death (at 3 years, 8.8% vs. 8.5%) and MACE (at 3 years, 12.0% vs. 11.5%). When the patients were divided into PCI-treated and CABG-treated patients according to the revascularization procedures, there were also no significant differences in the unadjusted incidence of all-cause death or MACE between women and men (**Figure 2**). **Table 4** shows the 3-year unadjusted outcomes in detail in the female vs. male patients. Female gender was associated with fewer unadjusted incidences of sudden death, although the numbers of cumulative events at 3 years were small in both groups (25 events, 1.0% in women; 98 events, 1.6% in men).

The results of a multivariate Cox proportional hazards model (**Table S1**) indicated that female gender was associated with a significantly lower adjusted risk of all-cause death (relative risk, 0.86; 95%CI, 0.77–0.96; P=0.005). BMI>25 and uses of anti-platelet drugs and statins at discharge were also included in the significant predictors of better survival. The independent predictors of all-cause mortality in our analyses were age ≥75 years, DM, history of heart failure, history of peripheral vascular disease, CKD, anemia, current smoker, and multivessel disease.

Coronary Revascularization During Follow-up

The incidence of any revascularization was significantly lower in women than in men (at 3 years, 28.2% vs. 31.2%, log-rank P=0.0037; **Table 4**, **Figure 3A**). The difference was mostly derived from the significantly lower incidence of

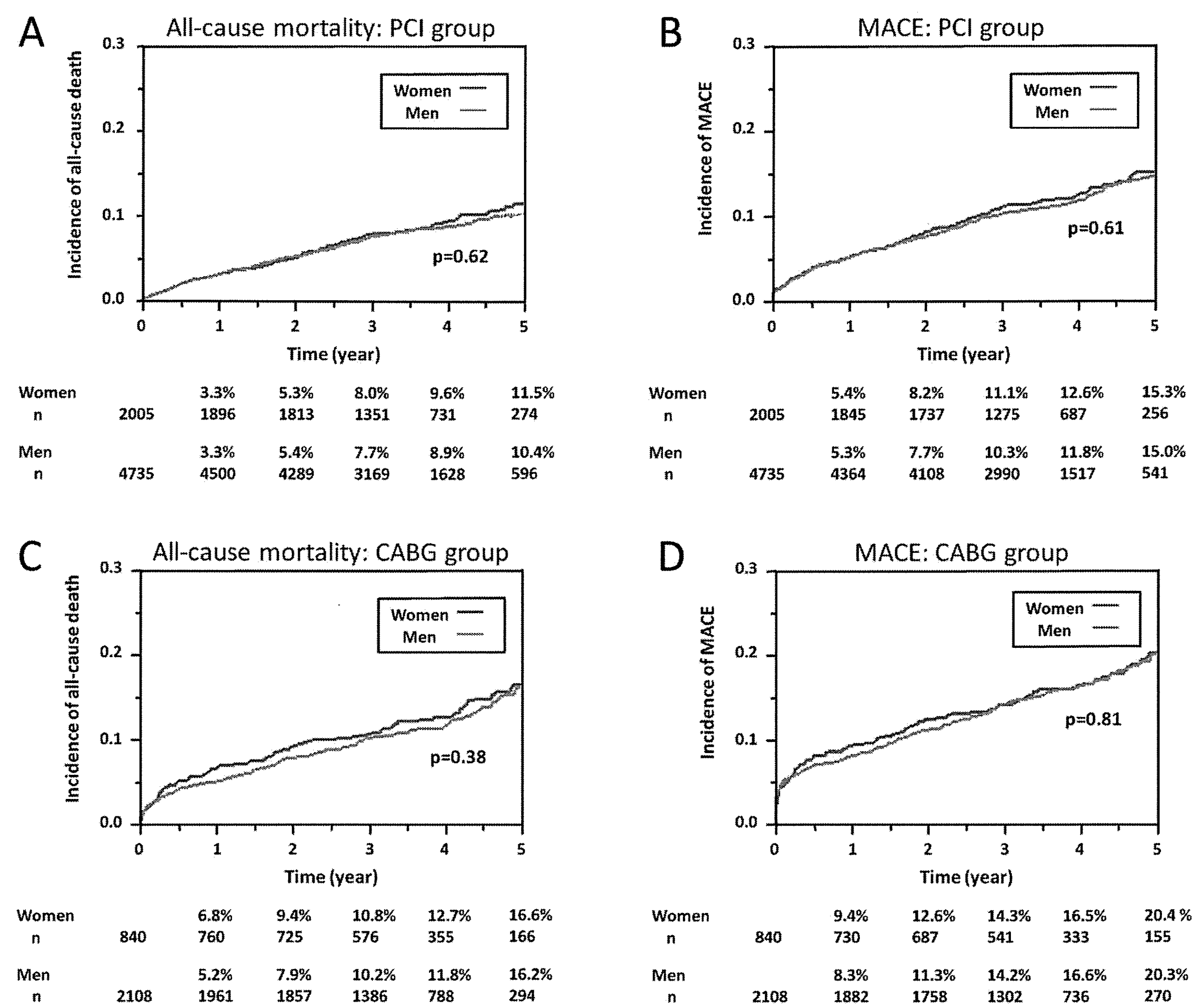

Figure 2. Incidence of all-cause death and major adverse cardiovascular events (MACE) in the percutaneous coronary intervention (PCI)-treated or coronary artery bypass graft (CABG)-treated patients. There were no significant differences between women (red lines) and men (green lines) in the unadjusted long-term incidence of all-cause death or MACE both in the PCI-treated and the CABG-treated subgroups. (**A**) Incidence of all-cause death in the PCI-treated patients. (**B**) Incidence of MACE in the PCI-treated patients. (**C**) Incidence of all-cause death in the CABG-treated patients. (**D**) Incidence of MACE in the CABG-treated patients.

Table 4. Crude 3-Year Event Rates in Women vs. Men

Follow-up outcomes (at 3 years)	Women	Men	P value
All-cause death (%)	8.8	8.5	0.37
Cardiovascular death (%)	6.4	5.5	0.064
Cardiac death (%)	4.9	4.5	0.30
Sudden death (%)	0.96	1.6	0.031
MI (%)	3.4	3.3	0.96
Stroke (%)	4.3	5.0	0.055
MACE (%)	12.0	11.5	0.61
PCI (%)	26.2	29.0	0.006
CABG (%)	3.5	4.0	0.09
Any revascularization (%)	28.2	31.2	0.0037
TLR PCI (PCI group) (%)	24.9	28.9	0.002
Non-TLR PCI (PCI group) (%)	17.2	19.2	0.043

MACE is defined as the composite of cardiovascular death, MI, and stroke.
MACE, major adverse cardiovascular events; TLR, target lesion revascularization. Other abbreviations see in Tables 1,2.

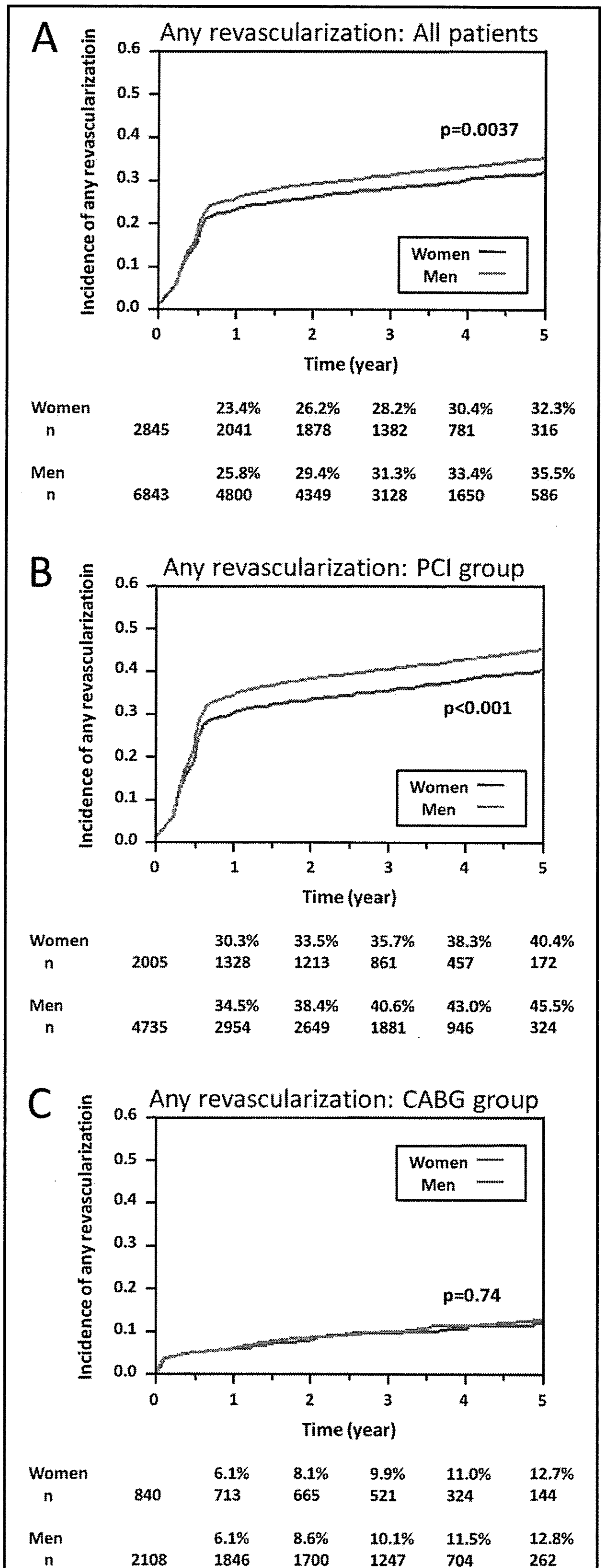

Figure 3. Incidence of any revascularization. The incidence of any revascularization was significantly lower in women (red lines) than in men (green lines) both in all patients and in the percutaneous coronary intervention (PCI)-treated patients. (**A**) All patients. (**B**) PCI-treated patients. (**C**) Coronary artery bypass graft (CABG)-treated patients.

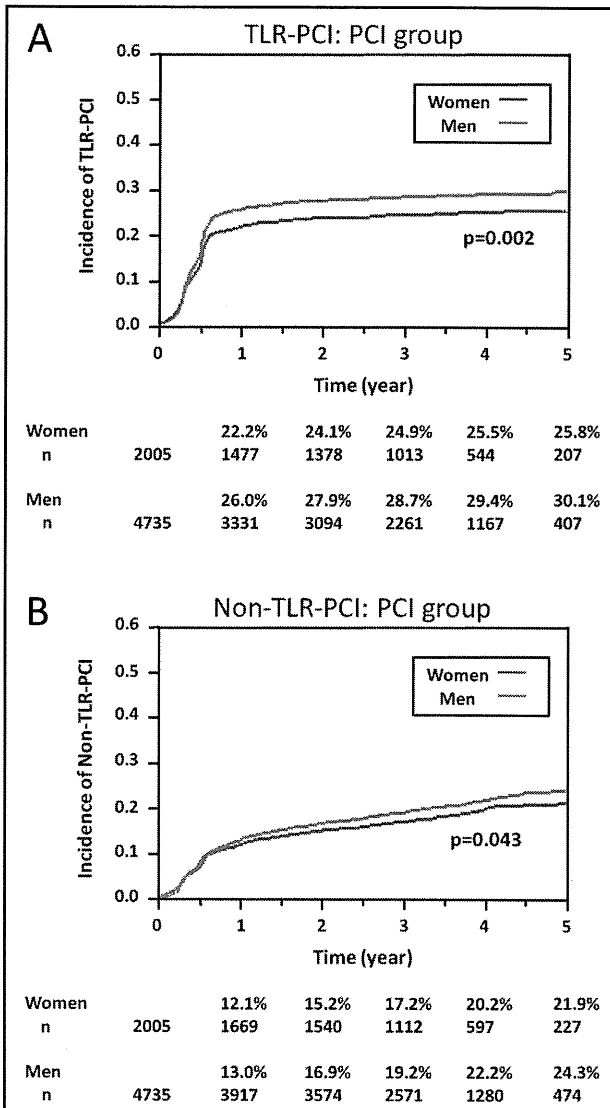

Figure 4. Incidence of target lesion revascularization (TLR)-percutaneous coronary intervention (PCI) and non-TLR-PCI in the PCI-treated patients. The significantly lower incidence in women (red lines) relative to men (green lines) was seen for both TLR-PCI and non-TLR-PCI. (**A**) TLR-PCI. (**B**) Non-TLR-PCI.

revascularization in women in the PCI-treated patients, and the incidence of coronary revascularization was comparable between women and men in the CABG-treated patients (**Figures 3B,C**). In the PCI-treated patients, the significantly lower incidence in women was seen both in target lesion revascularization (TLR)-PCI and in non-TLR-PCI (**Figure 4**). In a multivariate Cox proportional hazards model in all patients, DM, multivessel disease, use of nitrates at discharge, PCI as the revascularization procedure, and follow-up CAG were the independent predictors of any coronary revascularization during follow-up. Female gender was significantly associated with a lower incidence of any revascularization procedures, with a relative risk of 0.93 (95%CI, 0.88–0.99, P=0.014). Use of anti-platelet drugs at discharge was also associated with a lower incidence of any revascularization procedures (**Table S2**). In a multivariate analysis in the PCI-treated patients, female gender was also significantly associated with a lower incidence of any revascularization, with a relative risk of 0.92 (95%CI, 0.87–0.98, P=0.010). In addition, follow-up CAG was the strongest predictor of any coronary revascularization (**Table S3**).

Discussion

The present study has revealed that the prevalence of major coronary risk factors such as older age, DM, hypertension, hyper-LDL-cholesterolemia, and CKD were significantly higher in female Japanese patients undergoing coronary revascularization than in male patients. Despite the greater risk factor accumulation, the female gender conferred better long-term outcomes with regard to repeated coronary revascularization.

Baseline Characteristic Differences Between Female and Male Patients

Considering that the clinical presentation of CAD in women lagged 10 to 20 years behind men, it is not surprising that the women were approximately 5 years older than the men when the patients underwent first coronary revascularization. In addition, as previously reported,[2,7–10,12–14,21] women had higher incidences of coronary risk factors such as DM, hypertension, dyslipidemia, and CKD. Despite the greater risk factor accumulation including older age in women, the proportions of female and male patients with multivessel disease were similar in the present study. Female gender itself might play a protective role against the development of atherosclerotic diseases.

Revascularization Procedures and In-Hospital Outcomes

In the present study, in-hospital mortality and MACE were consistently comparable between women and men among all patients and among the PCI-treated or CABG-treated patients. In the PCI-treated patients there were no significant differences between women and men in terms of the ratios of stent use and the numbers of implanted stents per patient. Contrary to the similarities in the procedures for the PCI-treated patients, use of an ITA graft among the CABG-treated patients was less frequent in women than in men. The less frequent use of an ITA graft was consistent with previous observations,[10,14] and might be caused by smaller vessel sizes rather than the female gender itself.

It has been shown that in-hospital mortality is higher for women than men among patients undergoing CABG or PCI. In the 1985–1986 PTCA registry, in-hospital death was 10-fold higher in women than in men and female gender was an independent predictor of in-hospital death.[2] Similarly, a retrospective study in a cohort from 1985 to1993 found a higher rate of in-hospital death in women compared with men, and this difference remained statistically significant after adjustment for differences in baseline characteristics.[22] In patients undergoing CABG, 2.5-fold higher in-hospital mortality was reported in women relative to men.[23] The higher in-hospital mortality has been explained in part by advanced age, a higher prevalence of risk factors in women and difficulties associated with the procedures because of smaller physical and vessel sizes in women. In contrast to these studies, more recent studies have reported improved outcomes in women and described that greater experience as well as advances in technology and procedural techniques might contribute to more favorable outcomes in women.[9,10,13,14] In accordance with these reports, the present study showed comparable incidences of in-hospital death among women and men.

Previous studies have suggested the existence of gender-based differences in the treatment practices of CAD, which led to less intensive treatments for women than men.[24–26] Not only indication of invasive procedures but also the strategies of procedures such as PCI could differ by patients' gender. However, in the present study, there were no significant procedural differences between the women and men in the PCI-treated patients, for example, comparable numbers of used stents. Thus, our male and female patients appeared to be similarly treated in the index PCI procedures, and it is unlikely that the differences in the strategies of index PCI affected the differences in the outcomes between women and men. Although the use of an ITA graft was slightly less frequent in the CABG-treated women relative to men, this might be caused by the smaller vessel size in women, and the use of an ITA graft might not significantly affect the short-term in-hospital outcomes. Other techniques such as off-pump CABG might contribute to the improvement of in-hospital outcomes of both female and male patients and attenuate the gender-based differences.[27]

In the patients in the current study, the unadjusted incidence of in-hospital non-Q-wave myocardial infarction was significantly higher in female than male patients. However, the difference did not appear to critically affect the in-hospital as well as the long-term mortality.

Long-Term Outcomes in Women Relative to Men

Unadjusted survival analyses revealed that there were no significant differences according to gender with regard to all-cause death and MACE during long-term follow-up. After adjustment for possible confounding factors, including a higher baseline risk status in women, female gender was independently associated with a lower adjusted risk for mortality after revascularization. Moreover, better unadjusted survival free from any coronary revascularization was observed in female patients relative to male patients, and survival remained better in women after the adjustments.

There are several potential explanations for the possible differences in the long-term outcomes between female and male patients after revascularization. In the patients in the present study, women were treated more intensively with evidence-based secondary preventative medical therapies such as statins and ACEI/ARB. The more intensive use of medical therapies in women than in men might be rational in our patients, because greater coronary risk factor accumulation was seen in the female patients. A discrepancy exists between our observations and a previous report showing lower use of these medications in female patients with CAD.[28] As women have recently become more active in society, female CAD patients might become more intensively managed. Similar to the possible effects on in-hospital outcomes, improvements in technology and procedural techniques might contribute to improvement of the long-term outcomes of all CAD patients and attenuate the gender-based differences.

Although use of an ITA graft was a significant predictor of better long-term outcomes in patients undergoing CABG, the less frequent use of an ITA graft in female patients did not result in worse long-term outcomes in the present study. Better evidence-based medical treatment, especially therapy with statins, in female patients might have counteracted this disadvantage. It is also possible that the follow-up period was too short to reveal the effects of the difference in the use of an ITA graft between women and men.

The long-term survival rate free from any coronary revascularizations was significantly higher in women than in men in the present study, which was supported by the results of multivariate analyses indicating a significant association between female gender and lower incidence of revascularization. Because this difference by gender was mostly attributed to the difference in the PCI-treated patients and the survival curves began to separate at 6 months, the lower incidence of revascularization due to restenosis in women might cause this difference. There are several possible explanations for the lower incidence of restenosis-driven revascularization. If the female gender itself is an inhibitory factor for restenosis, the lower incidence of revascularization in women could be rational. It has been reported that sex hormones, especially estrogen, might prevent restenosis by attenuating the responses of vessel walls to injury.[29–31] However, the impact of sex hormones might be negligible in the present study because of the older age of the study patients. Thus, it is unlikely that the female gender conferred preventive effects for restenosis by the effects of sex hormones and overwhelmed the considerably increased restenosis risk by the smaller coronary artery diameters in women. Routine follow-up CAG might increase revascularization in patients with restenosis but without symptom and evidenced ischemia ("oculo-stenotic" reflex). Because follow-up CAG was less frequently performed in women, it might be possible that reduced non-clinically driven PCI resulted in the lower incidence of revascularization. In the present study, the rate of follow-up CAG after PCI was lower in women than in men. Women might be less willing to undergo invasive investigations than men,[32] and there might be more asymptomatic female patients who had restenosis but did not undergo revascularization. In addition, physician-based prejudices and gender-specific perception of angina symptoms might also be possible reasons.[33,34] As expected from previous studies,[19,20] follow-up CAG was a strong predictor of any revascularization procedure in the present study. However, after adjustment by the multivariate analysis, which included follow-up CAG as a variable, female gender still remained an independent predictor of freedom from any revascularization.

Study Limitations

Several limitations must be noted in addition to the limitations that are common to all observational studies caused by differences in patients' background characteristics among groups. Although angiographic findings such as vessel size, lesion length, stent length, and stent diameter might be associated with the occurrence of restenosis after PCI, such angiographic data were not available in this study cohort.

Data regarding blood tests, smoking habits, and medical therapies were only obtained at one time point, namely at hospitalization or discharge of the index hospitalization. Therefore, lifestyle modifications after revascularization such as discontinuation of smoking and improvement or deterioration of hypertension, DM and dyslipidemia, and adherence to medical therapies were not considered in the analyses.

Our database does not distinguish clinically driven PCI from non-clinically driven PCI. It is also impossible to distinguish scheduled routine follow-up CAG and follow-up CAG for symptomatic patients. Thus, the effects of follow-up CAG on the incidence of revascularization should be carefully interpreted, although the difference between men and women in the rate of follow-up CAG might be accounted for by scheduled routine follow-up CAG.

It could cause a critical bias to include follow-up CAG as a variable in the multivariate analysis for predictors of all-cause death. Because all patients who died before scheduled follow-up CAG were included in the patient group who did not undergo follow-up CAG and the total number of all-cause death might not be enough to dilute such a bias, the association of follow-up CAG or each of the other variables with the outcome might not be correctly assessed. Thus, follow-up CAG was not assessed in the multivariate analysis for possible predictors of all-cause death and MACE in the present study. Further studies designed to determine the long-term clinical benefits of follow-up CAG are needed.

Finally, in this observational study, indications for revascularization therapies were not defined but depended on the decision of each participating physician. Therefore, a gender-related selection bias could exist in the indications of treatment strategies between women and men. In addition, there are gender-related differences in angina symptoms and in the incidences of coronary spastic angina and microvascular angina that do not require revascularization.[35,36] Such differences can also affect the indications for CAG and coronary revascularization.

Conclusion

Because more modifiable coronary risk factors were accumulated in women who undergo coronary revascularization compared with men, more intensive management including evidence-based medications is required in female patients. However, the incidence of cardiovascular events was not higher in women than men and female gender was associated with a lower incidence of repeated revascularization relative to male gender in the era of bare metal stent use.

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Disclosure

The authors declare no conflict of interest.

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Appendix

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Supplemental Files

Supplemental File 1

Table S1. Multivariate Analyses for Predictors of All-Cause Death

Table S2. Multivariate Analyses for Predictors of Any Coronary Revascularization in All Patients

Table S3. Multivariate Analyses for Predictors of Any Coronary Revascularization in the PCI-Treated Patients

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Coronary Revascularization in Patients With Liver Cirrhosis

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Background. Liver cirrhosis is a major risk factor for cardiac surgery using cardiopulmonary bypass. However, percutaneous coronary intervention (PCI) or off-pump coronary artery bypass graft surgery (OPCABG) may be a less invasive alternative strategy.

Methods. Among the 9,877 patients undergoing first PCI or CABG enrolled in the CREDO-Kyoto Registry (a registry of first-time PCI and CABG patients in Japan), 332 patients diagnosed with liver cirrhosis were entered into the study (age 67.1 ± 9.4 years; 246 male). Liver cirrhosis was diagnosed by liver biopsy or signs of portal hypertension with characteristic morphologic liver and spleen changes.

Results. A total of 233 patients received PCI, 58 conventional on-pump CABG (CCABG), and 41 OPCABG. Median follow-up was 3.3 years. The PCI group included less complex coronary lesions such as triple vessel and left main disease ($p < 0.01$ each). Propensity score adjusted in-hospital mortality after CCABG or OPCABG was higher than that after PCI; however, the differences were not significant (odds ratio [95% confidence interval]: 6.84 [0.52 to 90.8], $p = 0.14$ for CCABG versus PCI; and 1.86 [0.08 to 45.8], $p = 0.71$ for OPCABG versus PCI). Adjusted overall mortality after CCABG or CABG was lower than that after PCI, but the differences were not significant (0.66 [0.31 to 1.40], $p = 0.28$; and 0.64 [0.28 to 1.49], $p = 0.31$, respectively). Approximately two thirds of patients died of noncardiovascular morbidities (malignancies, including hepatocarcinoma, or hepatic decompression).

Conclusions. Because overall noncardiovascular mortality is high among patients with liver cirrhosis, complete revascularization may not be associated with better survival outcomes. Further study is warranted to determine the impact of a coronary revascularization strategy for liver cirrhosis patients.

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Liver cirrhosis (LC) is one of the major causes of morbidity and mortality and is regarded as an increased risk factor for hepatic decompression after surgery [1]. It has also been shown to be a major risk factor for cardiac surgery particularly when using cardiopulmonary bypass [2, 3]. However, to date, only a few studies based on a small number of patients with mixed surgical procedures have been performed [2-9]. In addition, most of these reports included only in-hospital or short follow-up periods. The prognosis of patients with LC is generally poor owing to noncardiovascular disorders such as hepatic decompression. Thus, to determine an effective revascularization strategy for patients with LC, it is important to investigate not only cardiovascular but also noncardiovascular outcomes with a longer follow-up period.

Off-pump coronary artery bypass graft surgery (OPCABG) has been developed to reduce the risk of cardiopulmonary bypass [10]. Furthermore, percutaneous coronary intervention (PCI) may be a less invasive alternative strategy for patients with LC, particularly for those with less complex coronary lesions. The Coronary Revascularization Demonstrating Outcome Study in Kyoto (CREDO-Kyoto) is a multicenter registry from 30 institutions in Japan enrolling consecutive patients undergoing their first PCI or CABG [11]. In the present study, we show the current state of coronary revascularization strategies for LC patients in Japan, and analyze the outcomes of each revascularization strategy using the data from the registry.

Patients and Methods

Study Population

This study was approved by the Institutional Review Board or Ethics Committee of all participating institutions. Because the study subjects were retrospectively

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enrolled, written informed consent was not obtained, in concordance with the guidelines for epidemiologic studies issued by the Ministry of Health, Labor, and Welfare of Japan. However, 73 patients were excluded because of their refusal to participate in the study when contacted for follow-up [11].

Between January 2000 and December 2002, 9,877 patients were identified as having undergone either CABG or PCI without prior history of coronary revascularization. Among these, 332 patients who were diagnosed with LC were the subjects of the present study.

Data Collection and Definitions

Demographic, angiographic, and procedural data were collected from hospital charts or databases in each center by independent clinical research coordinators according to prespecified definitions. Follow-up data were obtained from hospital charts or by contacting patients or referring physicians.

A diagnosis of LC was made either by liver biopsy or signs of portal hypertension with characteristic morphologic changes in the liver and spleen confirmed by ultrasonography, computed tomography, and magnetic resonance imaging [5]. Other diagnostic criteria, such as varices, thrombocytopenia, ascites, encephalopathy, and biological abnormalities, were also employed. Left ventricular ejection fraction (LVEF) was measured either by contrast left ventriculography or by echocardiography. Chronic kidney disease was regarded as present when creatinine clearance estimated by the Cockcroft-Gould formula was less than 60 mL/min. Anemia was defined as a blood hemoglobin level less than 12 g/dL, as previously described [11].

Endpoints

An independent clinical events committee adjudicated events. Death was regarded as cardiovascular in origin unless obvious noncardiovascular causes could be identified. (Any death during the index hospitalization was regarded as cardiovascular death.) Myocardial infarction was adjudicated according to the definition in the Arterial Revascularization Therapy Study [12]. Within 1 week of the index procedure, only Q-wave myocardial infarctions were adjudicated as myocardial infarctions. Stroke at follow-up was defined as symptomatic stroke. The primary endpoint was death from any cause. Secondary endpoints were cardiovascular death, myocardial infarction, stroke, and the need for any revascularization procedures (PCI or CABG) during the follow-up period.

Table 1. Baseline Characteristics

	PCI n = 233	%	CCABG n = 58	%	OPCABG n = 41	%	p Value[a]
Age, years	67.0 ± 9.9		66.4 ± 8.4		68.4 ± 7.2		0.58
Male	171	73	46	79	29	71	0.57
Body mass index	23.5 ± 3.3		22.8 ± 2.9		22.6 ± 3.2		0.10
Emergency procedure	21	9	2	3	1	2	0.15
Ejection fraction	63.6 ± 11.6		58.8 ± 17.2		61.0 ± 14.8		0.05
Prior myocardial infarction	57	24	21	36	13	32	0.14
Heart failure	28	12	22	38	16	39	< 0.01
Atrial fibrillation	25	11	8	14	2	5	0.36
Stroke history	38	16	14	24	15	37	< 0.01
Peripheral vascular disease	17	7	10	17	13	32	< 0.01
Chronic pulmonary disease	3	1	1	2	3	7	0.045
Hypertension	166	71	35	60	31	76	0.19
Diabetes mellitus	99	42	32	55	25	61	0.04
Hyperlipidemia	80	34	24	41	19	46	0.27
Chronic kidney disease	73	31	22	38	15	37	0.56
Hemodialysis	21	9	6	10	4	10	0.95
Malignancy	35	15	6	10	5	12	0.62
Anemia	63	27	21	36	15	37	0.25
Current smoker	77	33	21	36	12	29	0.74
Coronary characteristics							
Number of diseased vessels	1.8 ± 0.8		2.3 ± 0.9		2.5 ± 0.8		< 0.01
Triple vessel disease	52	22	32	55	28	68	< 0.01
Left main disease	10	4	17	29	10	24	< 0.01
Proximal LAD disease	155	67	50	86	36	88	< 0.01
Total occlusion	66	28	20	34	19	46	0.06

[a] The p value is for comparison among percutaneous coronary intervention (PCI), conventional on-pump coronary artery bypass graft surgery (CCABG), and off-pump coronary artery bypass graft surgery (OPCABG).

LAD = left anterior descending artery.

Statistical Analyses

All continuous variables are expressed as the mean ± SD. Differences in baseline characteristics across the three groups were examined by analysis of variance of a χ^2 test. We used Kaplan-Meier estimates to plot survival curves in each group. The log rank test was used to identify significant differences in unadjusted survival curves.

Propensity scores, which represented the probabilities that a patient would undergo PCI, conventional on-pump CABG (CCABG), or OPCABG, were calculated for each patient. The propensity scores were estimated separately using multivariable logistic regression analyses. Confounding factors in the logistic regression included age, sex, body mass index, emergency procedure, prior myocardial infarction, congestive heart failure, stroke, peripheral arterial disease, atrial fibrillation, chronic obstructive pulmonary disease, malignancy, hypertension, diabetes mellitus, hemodialysis, chronic kidney disease, anemia, current smoker status, LVEF, total occlusion, proximal left anterior descending artery (LAD) disease, triple vessel disease, and left main disease. Outcomes after PCI, CCABG, or OPCABG were compared using the Cox proportional hazard models stratified by the quartiles of propensity scores. Propensity score adjusted hazard ratios, 95% confidence intervals, and *p* values are reported. The *p* values for multiple comparisons among the three groups were adjusted by the Bonferroni correction. All reported *p* values were two-sided.

To determine the baseline predictive factors for mortality, other than revascularization modality, we used the same potential variables above. Those variables for which *p* values were less than 0.05 in univariate analysis were included in the multivariate Cox proportional hazard model.

All analyses were conducted by a statistician using SAS software version 9.2 (SAS Institute, Cary, NC) and S-Plus version 7.0 (Insightful Corp, Seattle, WA), and all reported *p* values were two-sided. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the contents of the manuscript as written.

Results

Baseline Characteristics

Among the 332 patients diagnosed with LC, 233 patients (70.1%) were treated with PCI, 58 (14.51%) with CCABG, and 41 (12.4%) with OPCABG. Baseline characteristics of the patients in the three groups are shown in Table 1. Age, ratio of emergency procedure, and ejection fraction did not differ among the groups. Comorbidities such as malignancy and hemodialysis also did not differ among the groups. The PCI group generally included less high risk patients, such as those with diabetes and left ventricular dysfunction. The numbers of diseased vessels were lower in the PCI group. The PCI group included less complex patients, such as those with left main and proximal LAD disease. In the PCI group, bare-metal stents were used in 79% of patients. None of the patients received drug-eluting stents.

Operative outcomes are shown in Table 2. The number of diseased vessels and anastomotic sites did not differ between CCABG and OPCABG patients. More left and bilateral internal thoracic artery grafts were used in the OPCABG group. Concomitant operations were more common in the CCABG group.

In-Hospital and Follow-Up Outcomes

Clinical follow-up was 100% during the entire study period. The median follow-up period was 1,214 days for

Table 2. Operative Outcomes

	CCABG n = 58	%	OPCABG n = 41	%	*p* Value
Number of diseased vessels	2.3 ± 0.9		2.5 ± 0.8		0.20
Number of anastomotic sites	3.0 ± 1.1		3.3 ± 1.6		0.21
Preoperative IABP use	1	1.7	1	2.4	0.91
Emergency	2	3.4	1	2.4	0.77
Type of bypass graft					
Left internal thoracic artery	51	87.9	40	97.6	0.08
Bilateral internal thoracic artery	8	13.8	18	43.9	< 0.01
Right gastroepiploic artery	8	13.8	6	14.6	0.91
Radial artery	19	32.8	12	29.3	0.71
Saphenous vein	40	69.0	22	53.7	0.12
Concomitant surgery					
Aortic valve	5	8.6	0	0.0	
Mitral valve	5	8.6	0	0.0	
Thoracic aortic	2	3.4	0	0.0	
Abdominal/peripheral vascular	0	0.0	4	9.8	
Others	4	6.9	0	0.0	
Concomitant surgery total	16	27.6	4	9.8	0.03

CCABG = conventional on-pump coronary artery bypass graft surgery; IABP = intraaortic balloon pump; OPCABG = off-pump coronary artery bypass graft surgery.

the PCI group and 1,168 days for the CABG group. Regarding in-hospital outcomes, 1 patient in the PCI group, 4 in the CCABG group, and 1 in the OPCABG group died in the hospital (Table 3). Bleeding complications, such as postprocedure tamponade were more common in the CCABG group. During follow-up, 21 patients died of cardiovascular events, and 40 patients died of noncardiovascular events. Approximately two thirds of patients died of noncardiovascular events. Of the 40 patients who died of noncardiovascular events, 26 patients (65%) died of malignancy, including hepatocarcinoma or hepatic failure, during the follow-up period.

Survival Analyses

KAPLAN-MEIER ANALYSIS. Unadjusted freedom from all-cause death values of all patients at 30 days, 1 year, and 3 years were 98.5%, 93.9%, and 81.1%, respectively (Fig 1A). Freedom from all-cause death values did not differ between PCI and CABG groups (p = 0.34, Table 1). Freedom from all-cause and cardiovascular death values did not differ among PCI, CCABG, and OPCABG groups (p = 0.61 in Fig 1C, and p = 0.48 in Fig 1D).

PROPENSITY SCORE ANALYSIS. Propensity score adjusted in-hospital mortality did not differ between PCI and CABG groups (Table 4). There were also no significant differences in adjusted in-hospital mortality among PCI, CCABG, and OPCABG groups. Similarly, adjusted overall mortality did not differ between PCI and CABG groups (Table 4). There were no significant differences in adjusted overall mortality among the three groups.

RISK FACTORS FOR MORTALITY. The strongest predictive variable for overall all-cause death was comorbid malignancy (Table 5). Significant predictive variables for cardiovascular death were hemodialysis, heart failure, stroke history, anemia, triple vessel disease, and left main disease (Table 5).

Comment

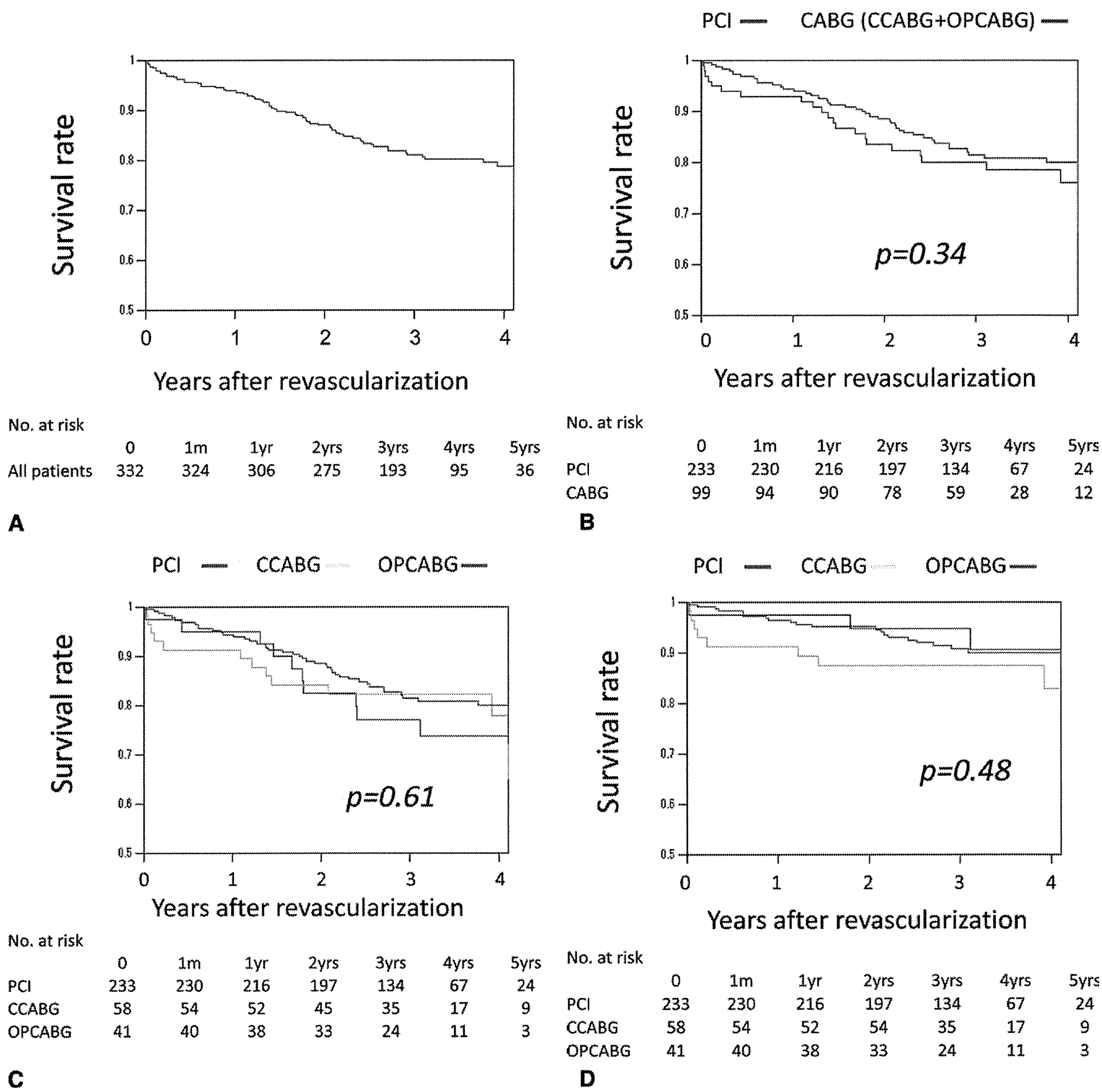
Main Findings

In the present study, we show the current state of coronary revascularization strategies for LC patients in Japan, and analyze the outcomes of each revascularization strategy. We obtained the following findings: (1)

Table 3. In-Hospital and Follow-Up Outcomes

	PCI		CCABG		OPCABG		Total	
In-hospital outcomes	(n =233)		(n = 58)		(n = 41)		(n = 332)	
Death								
Myocardial infarction	1	0.4%	1	1.7%	0	0.0%	2	0.6%
Renal failure	0	0.0%	1	1.7%	0	0.0%	1	0.3%
Sepsis	0	0.0%	1	1.7%	1	2.4%	2	0.6%
Bleeding	0	0.0%	1	1.7%	0	0.0%	1	0.3%
Death total	1	0.4%	4	6.9%	1	2.4%	6	1.8%
Events								
Stroke	0	0.0%	1	1.7%	2	4.9%	3	0.9%
Myocardial infarction	9	3.9%	0	0.0%	2	4.9%	11	3.3%
Bleeding	1	0.4%	5	8.6%	2	4.9%	8	2.4%
Follow-up outcomes	(n = 232)		(n = 54)		(n = 40)		(n = 326)	
Death								
Cardiovascular	16	6.9%	3	5.6%	2	5.0%	21	6.4%
Noncardiovascular								
Hepatic failure	0	0.0%	1	1.9%	1	2.5%	2	0.6%
Hepatocarcinoma	7	3.0%	2	3.7%	2	5.0%	11	3.4%
Other carcinomas	11	4.7%	0	0.0%	2	5.0%	13	4.0%
Renal failure	2	0.9%	1	1.9%	0	0.0%	3	0.9%
Pneumonia	0	0.0%	1	1.9%	1	2.5%	2	0.6%
Sepsis	3	1.3%	0	0.0%	0	0.0%	3	0.9%
Gastrointestinal	2	0.9%	1	1.9%	0	0.0%	3	0.9%
Unknown	2	0.9%	0	0.0%	1	2.5%	3	0.9%
Noncardiovascular total	27	11.6%	6	11.1%	7	17.5%	40	12.3%
Death total	43	18.5%	9	16.7%	9	22.5%	61	18.7%
Events								
Stroke	9	3.9%	4	7.4%	1	2.5%	14	4.3%
Myocardial infarction	10	4.3%	2	3.7%	0	0.0%	12	3.7%
Revascularization	95	40.9%	7	13.0%	5	12.5%	107	32.8%

CCABG = conventional on-pump coronary artery bypass graft surgery; OPCABG = off-pump coronary artery bypass graft surgery; PCI = percutaneous coronary intervention.



A — No. at risk

	0	1m	1yr	2yrs	3yrs	4yrs	5yrs
All patients	332	324	306	275	193	95	36

B — No. at risk

	0	1m	1yr	2yrs	3yrs	4yrs	5yrs
PCI	233	230	216	197	134	67	24
CABG	99	94	90	78	59	28	12

C — No. at risk

	0	1m	1yr	2yrs	3yrs	4yrs	5yrs
PCI	233	230	216	197	134	67	24
CCABG	58	54	52	45	35	17	9
OPCABG	41	40	38	33	24	11	3

D — No. at risk

	0	1m	1yr	2yrs	3yrs	4yrs	5yrs
PCI	233	230	216	197	134	67	24
CCABG	58	54	52	54	35	17	9
OPCABG	41	40	38	33	24	11	3

Fig 1. (A) Freedom from all-cause death (all patients). (B) Freedom from all-cause death, percutaneous coronary intervention (PCI [red line]) versus coronary artery bypass graft surgery (CABG [blue line]). (C) Freedom from all-cause death, PCI (red line) versus conventional on-pump CABG (CCAB [green line]) versus off-pump CABG (OPCAB [blue line]). (D) Freedom from cardiovascular death, PCI (red line) versus CCAB (green line) versus OPCAB (blue line).

Compared with PCI, CABG was applied to patients with more complex coronary lesions and more comorbidities, such as heart failure and diabetes. (2) Propensity score adjusted in-hospital morality after PCI tended to be low compared with that of CCABG or OPCABG. (3) Adjusted in-hospital mortality after OPCABG tended to be low compared with that of CCABG. (4) Adjusted overall all-cause mortality after CABG tended to be low compared with that of PCI. (5) Adjusted overall mortality was similar for CCABG and OPCABG. (6) Overall mortality was high regardless of revascularization strategy because of the high incidence of noncardiovascular death caused primarily by malignancy or hepatic decompression.

These results indicate that it is not possible to determine the optimal revascularization strategy for patients with LC from our registry because there were significant differences in the patient population for the three revascularization strategies, and noncardiovascular mortality was high. A randomized controlled trial may clarify the problem, but it is difficult to obtain matched patient populations because such patients are less common owing to varying degrees of severity of LC.

Table 4. Odds and Hazard Ratios for Death Comparing Each Revascularization Strategy By Propensity Score Stratification

	Propensity Score Adjusted		
	Odds Ratio	95% CI	p Value
In-hospital death			
CABG versus PCI	4.37	0.30 to 62.5	0.28
CCABG versus PCI	6.86	0.52 to 90.8	0.14
OPCABG versus PCI	1.86	0.08 to 45.8	0.71
CCABG versus OPCABG	3.70	0.33 to 40.7	0.29
Overall death			
CABG versus PCI	0.66	0.34 to 1.27	0.21
CCABG versus PCI	0.66	0.31 to 1.40	0.28
OPCABG versus PCI	0.64	0.28 to 1.49	0.31
CCABG versus OPCABG	1.03	0.43 to 2.44	0.95

CABG = coronary artery bypass graft surgery; CCABG = conventional on-pump coronary artery bypass graft surgery; CI = confidence interval; OPCABG = off-pump coronary artery bypass graft surgery; PCI = percutaneous coronary intervention.

Liver Cirrhosis and Cardiac Surgery

There have been several observational studies that investigated the outcomes of cardiac surgery in patients with LC [2–9]. Although these studies included a limited population with mixed surgical procedures, all these studies demonstrated that LC is a serious risk factor of cardiac surgery. The studies employed the Child-Turcotte-Pugh classification to evaluate the severity of LC [13, 14]. The operative mortality rate of class A patients was relatively low (0% to 10%) [2-9]. On the contrary, early studies reported higher mortality rates in class B patients, which ranged from 50% to 80% [2, 3]. Recent studies, with the exception of the report from Lin and colleagues [5], also reported relatively high mortality rates of 18% to 67% for group B patients as compared with class A patients [6–9]. However, all these studies involved in-hospital or short-term follow-up periods. The Model for End-Stage Liver Disease scoring system [15] has been validated for predicting survival in patients with end-stage liver disease. This score may be useful in predicting the prognosis of patients undergoing cardiac surgery [8, 9].

Benefit of Complete Revascularization for LC

Long-term survival outcomes in patients with LC were poor as compared with those of the whole population of the CREDO-Kyoto registry. Freedom from all-cause death values of the whole population at 30 days, 1 year, and 3 years were 99.4%, 96.6%, and 91.6% in the PCI group and 98.0%, 94.3%, and 89.3% in the CABG group, respectively; whereas, in the present study of patients with LC, freedom from all-cause death values at 30 days, 1 year, and 3 years were 99.6%, 99.4%, and 81.6% in the PCI group and 96.0%, 92.9%, and 80.1% in the CABG group, respectively. It is noteworthy that approximately two thirds of patients died of noncardiovascular morbidities such as malignancy, including hepatocarcinoma or hepatic failure, during follow-up. These results indicate that late mortality rather than early mortality was higher in patients with LC because noncardiovascular causes such as malignancy lower the chance of survival. This finding might mean that complete revascularization may not contribute to improving long-term outcome.

Coronary Revascularization and LC

Regardless of recent decreases in overall perioperative mortality, postoperative morbidities remain a major issue in this population. It is well known that cardiopulmonary bypass triggers production and release of numerous vasoactive substances and cytotoxic mediators that affect coagulopathy, the immune system, vascular resistance, vascular permeability, fluid balance, and major organ functions [7]. Therefore, OPCABG may be advantageous in avoiding such perioperative complications. Hayashida and associates [6] reported that no patient with the Child-Turcotte-Pugh classification class B undergoing OPCABG died in the hospital. Filsoufi and coworkers [8] also reported that no mortality occurred among patients who underwent OPCABG.

Based on these findings, we suggest the following

Table 5. Predictive Variables[a] for All-Cause Death and Cardiovascular Death

	Multivariate Hazard Ratio	95% CI	p Value
All-cause death			
Malignancy	3.26	1.86 to 5.75	< 0.01
Left main disease	2.11	1.09 to 4.08	0.03
Hemodialysis	2.03	0.99 to 4.18	0.05
Diabetes mellitus	1.88	1.11 to 3.19	0.02
Triple vessel disease	1.60	0.96 to 2.68	0.07
Age, per years old	1.03	0.99 to 1.06	0.12
Anemia	1.49	0.83 to 2.69	0.18
Stroke history	1.38	0.78 to 2.43	0.27
Chronic total occlusion	1.36	0.81 to 2.29	0.25
Heart failure	1.12	0.61 to 2.07	0.71
Cardiovascular death			
Hemodialysis	4.65	3.12 to 6.94	< 0.01
Heart failure	1.56	1.14 to 2.12	< 0.01
Stroke history	1.55	1.12 to 2.15	< 0.01
Anemia	1.55	1.12 to 2.15	< 0.01
Triple vessel disease	1.49	1.05 to 2.13	0.03
Left main disease	1.47	1.05 to 2.06	0.02
Peripheral artery disease	1.27	0.91 to 1.78	0.17
Diabetes mellitus	1.02	0.76 to 1.37	0.90

[a] These variables were selected from the following potential variables (univariate $p < 0.05$): age, sex, body mass index, emergency procedure, prior myocardial infarction, congestive heart failure, stroke, peripheral arterial disease, atrial fibrillation, chronic obstructive pulmonary disease, malignancy, hypertension, diabetes mellitus, hemodialysis, chronic kidney disease, anemia, current smoker status, left ventricular ejection fraction, total occlusion, proximal left anterior descending artery disease, triple vessel disease, and left main disease.

CI = confidence interval.

revascularization strategy: because long-term outcomes are largely influenced by noncardiovascular events in LC patients, PCI, rather than complete revascularization (by CCABG or OPCABG), should be recommended for patients with complex coronary lesions accompanied by malignancies or severe hepatic decompression as well as for patients with less complex coronary lesions. If CABG is indicated, CCABG or OPCABG should be selected based on the balance between the patient's general condition and the need for complete revascularization, because complete revascularization may not be associated with better survival outcomes. However, further study is warranted to determine the impact of a coronary revascularization strategy for LC patients.

Study Limitations

There are several important limitations to the present study, the most important being that the CREDO-Kyoto registry lacks the preoperative data of hepatic function, such as bilirubin and aspartate aminotransferase. The severity of liver disease could not be determined by the Child-Turcotte-Pugh classification or the Model for End-Stage Liver Disease score. These data may influence the outcomes of multivariate analyses. Second, several biases may exist, such as indications regarding the revascularization strategies and level of expertise in the procedures for each institution and physician involved in the registry. Finally, since our study is nonrandomized, these potential confounders may influence our results.

In conclusion, because overall noncardiovascular mortality (eg, malignancy or hepatic decompression) is high among patients with LC, complete revascularization may not necessarily be associated with better survival outcomes. The revascularization modality should be selected after consideration of the balance between the patient's general condition and the severity of coronary lesions. Further study is warranted to determine the impact of the different coronary revascularization strategies on long-term outcomes for patients with LC.

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Oral pretreatment with a green tea polyphenol for cardioprotection against ischemia–reperfusion injury in an isolated rat heart model

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Objective: Ischemia–reperfusion injury is among the most serious problems in cardiac surgery. Epigallocatechin-3-gallate, a major polyphenolic component of green tea, is thought to be cardioprotective through its antioxidant activities. We investigated cardioprotective effects of oral epigallocatechin-3-gallate pretreatment against ischemia–reperfusion injury in isolated rat hearts and considered possible underlying mechanisms.

Methods: Rats were given epigallocatechin-3-gallate solution orally at 0.1, 1, or 10 mmol/L (n = 12 per group) for 2 weeks; controls (n = 12) received tap water alone for 2 weeks. Subsequently, Langendorff-perfused hearts were subjected to global ischemia for 30 minutes, followed by 60 minutes of reperfusion.

Results: Recoveries at 60 minutes after reperfusion of left ventricular developed pressure and maximum positive and minimum negative first derivatives of left ventricular pressure were significantly higher in 1-mmol/L group than in 0.1-mmol/L ($P < .0001$), 10-mmol/L ($P < .05$), and control ($P < .0001$) groups. Oxidative stress after reperfusion, as reflected by 8-hydroxy-2′-deoxyguanosine index, was lower in 1-mmol/L group than in control ($P < .01$) and 0.1-mmol/L ($P < .05$) groups. Western blot analysis after reperfusion showed p38 activation and active caspase-3 expression to be lower in 1-mmol/L group than in control group ($P < .05$).

Conclusions: Oral pretreatment with epigallocatechin-3-gallate preserved cardiac function after ischemia–reperfusion, an effect that may involve its antioxidative, antiapoptotic properties, although a high dose did not lead to dramatic improvement in cardiac function. Oral epigallocatechin-3-gallate pretreatment may be a novel and simple cardioprotective method for preventing perioperative cardiac dysfunction in cardiac surgery. (J Thorac Cardiovasc Surg 2011;141:511-7)

Ischemia–reperfusion injury (IRI) remains among the most serious problems in cardiac surgery. Several mechanisms and mediators of IRI have been described.[1] Reactive oxygen species reportedly play an important role in the pathogenesis of myocardial IRI.[2,3] Administrations of various free radical scavengers and exogenous antioxidants have been demonstrated to reduce myocardial IRI and improve cardiac function in animal models.[4,5]

Epigallocatechin-3-gallate (EGCG) is well known to be the most abundant polyphenolic catechin in green tea. Green tea is safe, popular, and known for its antioxidant properties, mainly through scavenging of reactive oxygen species (eg, superoxide anion, hydrogen peroxide, and hydroxyl radical).[6] Several studies have shown green tea consumption to have potential protective effects against cardiovascular disease,[7,8] thought to be attributable to its antioxidant activities.[9] Little is known, however, about the cardioprotective effects of preoperative oral intake of green tea polyphenols against ischemia followed by reperfusion of the heart.

We previously demonstrated, with a Langendorff-perfused rat heart model, that several exogenous antioxidants may enhance recovery after myocardial IRI by diminishing oxidative stress.[10,11] In this study, we evaluated the significance of the cardioprotective effects against myocardial IRI of oral pretreatment with a green tea polyphenol and examined its dose effects, as well as the possible underlying mechanisms, in an isolated rat heart model.

ET/BS

MATERIALS AND METHODS

Green Tea Polyphenol Solutions

Green tea extract in pure form of EGCG was purchased from DSM Nutritional Products Ltd (Basel, Switzerland). EGCG solutions at concentrations of 1 mmol/L, 0.1 mmol/L, and 10 mmol/L were made with drinking water.

Animals

Male 11-week-old Sprague–Dawley rats (350–450 g body weight) were used for this study. All animals in this study received humane care in compliance with 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 prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health, (www.nap.edu/catalog/5140.html).

Study Groups

The rats were randomly divided into 4 groups of 12 animals each. In the 0.1-mmol/L, 1-mmol/L, and 10-mmol/L EGCG groups, rats were allowed

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Abbreviations and Acronyms

8-OHdG	= 8-hydroxy-2′-deoxyguanosine
dp/dt	= first derivative of left ventricular pressure
EGCG	= epigallocatechin-3-gallate
HR	= heart rate
IRI	= ischemia–reperfusion injury
LV	= left ventricle
MAPK	= mitogen-activated protein kinase

to drink the respective EGCG solutions as their daily fluid intakes for 2 weeks. In the control group, rats drank regular water for 2 weeks. Fluid consumption was measured daily for each rat.

Measurement of Plasma EGCG Levels

A technique of liquid chromatography coupled with tandem mass spectrometry was used to measure EGCG in rat plasma as previously described.[12] Just before the Langendorff study, a blood sample was obtained from the inferior vena cava of each rat that had been given EGCG for 2 weeks. Samples were centrifuged at 3000 g for 10 minute. The resulting plasma (100 μL) was mixed with 20 μL of an internal standard (gallocatechin gallate, 0.6 μg/mL) and 20 μL buffer solution (2% ascorbic acid and 1-mol/L sodium acetate, pH 5.0). The mixture was then extracted with 100 μL of a methanol and acetic acid mixture (100/5 volume/volume) and centrifuged for 15 minutes at 11750*g*. After centrifugation, 100 μL of the clear supernatant were transferred to another centrifuge tube, dried under nitrogen, and reconstituted with 100 μL of mobile phase A. An aliquot of the solution was directly injected onto the setup for liquid chromatography coupled with tandem mass spectrometry for analysis, and the plasma EGCG concentration was calculated.

Heart Isolation and Perfusion

Isolated hearts were perfused with a Langendorff apparatus as previously described.[10] The rats were anesthetized by inhalation of diethyl ether and intraperitoneal injection of sodium pentobarbital (50 mg/kg). Anticoagulation was achieved with an intravenous injection of heparin (1000 IU/kg). Each heart was quickly excised, washed in ice-cold saline solution, and mounted on a nonrecirculating Langendorff apparatus. After cannulation of the aorta, the coronary circulation was quickly restarted at a constant pressure of 80 mmHg at 37°C. Krebs–Henseleit solution (sodium chloride 118 mmol/L, potassium chloride 4.7 mmol/L, magnesium sulfate 1.2 mmol/L, sodium hydrogen carbonate 25 mmol/L, monobasic potassium phosphate 1.2 mmol/L, calcium chloride 2.5 mmol/L, and glucose 11 mmol/L) bubbled with a mixture of 95% oxygen and 5% carbon dioxide was used for perfusion. For measurement of left ventricular (LV) function, a water-filled latex balloon was inserted through the left atrium into the LV after a pulmonary arteriotomy. The balloon pressure was adjusted to maintain LV end-diastolic pressure at 10 mm Hg. After a stabilization period of at least 20 minutes, the isolated heart was subjected to no-flow global ischemia at 37°C for 30 minutes, followed by 60 minutes of reperfusion. The LV pressure waveform was traced on a strip chart.

Evaluation of LV Function

To measure LV pressure, the latex balloon was connected by a fluid-filled polyethylene tube to a pressure transducer. After achievement of preischemic perfusion equilibrium, heart rate (HR), LV developed pressure, and positive and negative first derivatives of LV pressure (+dp/dt and −dp/dt, reflecting both systolic and diastolic function) were measured and recorded continuously with LV diastolic pressure stabilized at 10 mm Hg. Coronary flow was measured by collecting coronary effluent buffer. LV function was assessed by calculating the percentage recovery of each parameter (eg, % recovery of HR = HR after 60 minutes of reperfusion/baseline HR × 100%).

Immunohistochemical Assay for Evaluation of Oxidative Stress

The level of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a major product of oxidative DNA modifications,[13] is widely used as a marker of oxidative stress against DNA.[10,14] Cardiac tissues were fixed overnight in paraformaldehyde phosphate buffer solution immediately after reperfusion and then dehydrated sequentially with 50% and 70% ethanol for 24 hours. For immunohistochemical analysis, the avidin–biotin complex method was carried out as previously described.[14] Briefly, after deparaffinization of the specimens, appropriately diluted solutions of normal rabbit serum (Dako Japan Co, Ltd, Kyoto, Japan), mouse monoclonal antibody against 8-OHdG (Japan Institute for the Control of Aging, Fukuroi, Japan), biotin-labeled rabbit anti–mouse IgG serum (Dako), and avidin–biotin complex (Vector Laboratories, Inc, Burlingame, Calif) were sequentially applied. The substrate for alkaline phosphatase (black) was obtained from a vector.

Immunohistologic data (8-OHdG index) were quantified as follows[15]: 8-OHdG index = Σ[(X − threshold) × area (pixels)]/total cell number, where *X* is the staining density indicated in gray scale with ImageJ (National Institutes of Health, http://rsbweb.nih.gov/ij/) and Photoshop (Adobe Systems Inc, San Jose, Calif) software.

Western Blotting Analysis for Apoptosis-Related Proteins

Western blotting was performed to identify apoptosis in the myocardium at 60 minutes after reperfusion, as previously described.[16] Ventricular tissue samples obtained from ischemic, reperfused hearts (n = 5 per group) were homogenized on ice in a lysis buffer with protease inhibitor and 1-mmol/L orthovanadic acid and quantified for protein levels with a commercially available assay (BCA protein assay reagent kit; Thermo Fisher Scientific Inc, Rockford, Ill). Proteins (20 μg/sample) were separated with sodium dodecylsulfate polyacrylamide gels (20%) and electrotransferred onto nitrocellulose membranes (Immobilon P; Millipore, Billerica, Mass). After blocking with 5% nonfat milk in tris(hydroxymethyl)aminomethane-buffered saline solution containing 0.1% polysorbate 20, membranes were incubated overnight with the following first antibodies: (1) p38 kinase (sc-7972) at a 1:1000 dilution, (2) phosphorylated p38 (sc-7973) at a 1:1000 dilution, (3) caspase-3 (C9598) at a 1:1000 dilution, and (4) activated caspase-3 (C8487) at a 1:1000 dilution. After incubation with these primary antibodies, horseradish peroxidase–conjugated secondary antibodies were added (1:4000 dilution) for 1 hour at room temperature. Protein bands were enhanced with a chemiluminescence Western blotting determination kit (ECL-Plus; Amersham Pharmacia, Little Chalfont, UK). The band intensity was quantified with imaging software (ImageJ version 1.3).

Statistical Analysis

All data were expressed as mean ± SD. Statistical analyses were performed with statistical software (StatView for Windows version 5.0; SAS Institute Inc, Cary, NC). Data were analyzed by Student *t* test or 1-factor analysis of variance. When results were significant by analysis of variance, differences between individual groups were estimated with the Bonferroni–Dunn post hoc test.

RESULTS

Plasma EGCG Levels in Rats

In all groups, each rat received on average 30 mL/day of EGCG solution or tap water. As the dose of EGCG administered rose, plasma EGCG levels correspondingly increased

TABLE 1. Baseline parameters before ischemia

Variable	Control group (n = 12)	Epigallocatechin-3-gallate groups 0.1 mmol/L (n = 12)	1 mmol/L (n = 12)	10 mmol/L (n = 12)
Body weight (g)	422.8 ± 27.9	423.8 ± 19.2	420.1 ± 21.9	428.1 ± 19.1
Heart weight (g)	1.57 ± 0.08	1.61 ± 0.17	1.60 ± 0.14	1.62 ± 0.12
Heart weight/body weight (%)	0.37 ± 0.02	0.37 ± 0.03	0.38 ± 0.03	0.37 ± 0.03
Heart rate (beats/min)	315.4 ± 24.5	312.4 ± 20.4	326.0 ± 32.2	324.5 ± 38.4
LVDP (mm Hg)	96.6 ± 16.4	95.6 ± 11.6	98.5 ± 14.2	98.4 ± 14.3
Maximum +dp/dt (mm Hg/s)	2996.0 ± 538.2	2512.8 ± 301.7	3069.1 ± 727.3	2910.5 ± 654.3
Minimum −dp/dt (mm Hg/s)	2569.0 ± 514.8	2243.6 ± 267.7	2526.0 ± 450.0	2370.4 ± 425.6
Coronary flow (mL/min)	14.9 ± 3.8	16.8 ± 3.4	15.1 ± 3.3	17.1 ± 4.8

All values are mean ± SD. *LVDP*, Left ventricular developed pressure; *dp/dt*, first derivative of left ventricular pressure.

to a greater extent in rats given EGCG solution (0.1, 1, and 10 mmol/L) as drinking fluid for 14 days. Plasma EGCG was significantly higher in the 10-mmol/L group than in the control group (92.7 ± 29.8 vs 0 ng/mL, $P < .0001$), 0.1-mmol/L group (92.7 ± 29.8 vs 0 ng/mL, $P < .0001$), and 1-mmol/L group (92.7 ± 29.8 vs 6.2 ± 2.9 ng/mL, $P < .0001$). Plasma EGCG was undetectable in the 0.1-mmol/L group, just as in the control group.

Baseline Measurement Before Ischemia (Table 1)

There were no significant differences in body or heart weights before the Langendorff study among the 4 groups. Moreover, baseline LV function in the Langendorff study did not differ significantly among the groups.

Cardiac Function After Reperfusion

LV function measurements at 60 minutes after reperfusion are shown in Table 2. The percentage recoveries of LV developed pressure (Figure 1, *B*), maximum +dp/dt (Figure 1, *C*), and minimum –dp/dt (Figure 1, *D*) after 60 minutes of reperfusion were significantly higher in the 1-mmol/L and 10-mmol/L groups than in the control group. Among EGCG groups, the percentage recoveries of LV developed pressure (Figure 1, *B*), maximum +dp/dt (Figure 1, *C*), and minimum –dp/dt (Figure 1, *D*) were significantly higher in the 1-mmol/L group than in the 0.1-mmol/L and 10-mmol/L groups. There were no significant differences in HR among the 4 groups (Figure 1, *A*). Among all cardiac parameters, recovery after reperfusion in the 0.1-mmol/L group was almost equal to that in the control group. There were no significant differences in coronary flow at 60 minutes of reperfusion among the groups (Table 2).

Oxidative Stress on DNA

Representative myocardial images of 8-OHdG immunohistochemical staining are shown in Figure 2, *A*. In the hearts of the 1-mmol/L and 10-mmol/L group rats, there were fewer darkly stained nuclei than in the control and 0.1-mmol/L groups. As shown in Figure 2, *B*, the 8-OHdG index calculated from staining was significantly lower in the 1-mmol/L group than in the control group (98.2 ± 39.4 vs 244.5 ± 105.0 × 10^2, $P < .01$) and 0.1-mmol/L (98.2 ± 39.4 vs 221.9 ± 79.1 × 10^2, $P < .05$) groups. This index was also significantly lower in the 10-mmol/L group than in the control group (125.9 ± 83.2 vs 244.5 ± 105.0 × 10^2, $P < .05$).

Expression of Apoptosis-Related Proteins

Western blotting analyses for p38 (Figure 3, *A*) and caspase-3 (Figure 3, *B*) showed lower expressions of phosphorylated p38 and active caspase-3 in the 1-mmol/L group than in the control group ($P < .05$). Phosphorylation of p38 and caspase-3 cleavage were also significantly lower in the 10-mmol/L group than in the control group ($P < .05$).

DISCUSSION

We found oral pretreatment with EGCG to attenuate myocardial IRI and to preserve LV function after reperfusion in a Langendorff-perfused rat heart model. The antioxidative and antiapoptotic properties of EGCG may be involved in this cardioprotective effect. In addition, oral intake of

TABLE 2. Left ventricular function measurements at 60 minutes after reperfusion

Variable	Control group (n = 12)	Epigallocatechin-3-gallate groups 0.1 mmol/L (n = 12)	1 mmol/L (n = 12)	10 mmol/L (n = 12)
Heart rate (beats/min)	311.0 ± 46.1	301.6 ± 26.1	309.9 ± 22.3	298.4 ± 28.3
LVDP (mm Hg)	61.0 ± 17.4	59.3 ± 7.9	83.9 ± 12.8*†‡	72.3 ± 10.2§‖
Maximum +dp/dt (mm Hg/s)	1920.7 ± 591.0	1637.7 ± 174.8	2648.6 ± 599.5*‡	2262.0 ± 595.4‖
Minimum −dp/dt (mm Hg/s)	1589.2 ± 378.9	1403.1 ± 167.1	2033.1 ± 291.7*†‡	1745.0 ± 345.9‖
Coronary flow (mL/min)	10.5 ± 2.7	12.2 ± 1.9	13.0 ± 2.4	12.7 ± 3.3

All values are mean ± SD. *LVDP*, Left ventricular developed pressure; *dp/dt*, first derivative of left ventricular pressure. *$P < .01$ versus control. †$P < .01$ versus 0.1 mmol/L. ‡$P < .05$ versus 10 mmol/L. §$P < .05$ versus control. ‖$P < .05$ versus 0.1 mmol/L.

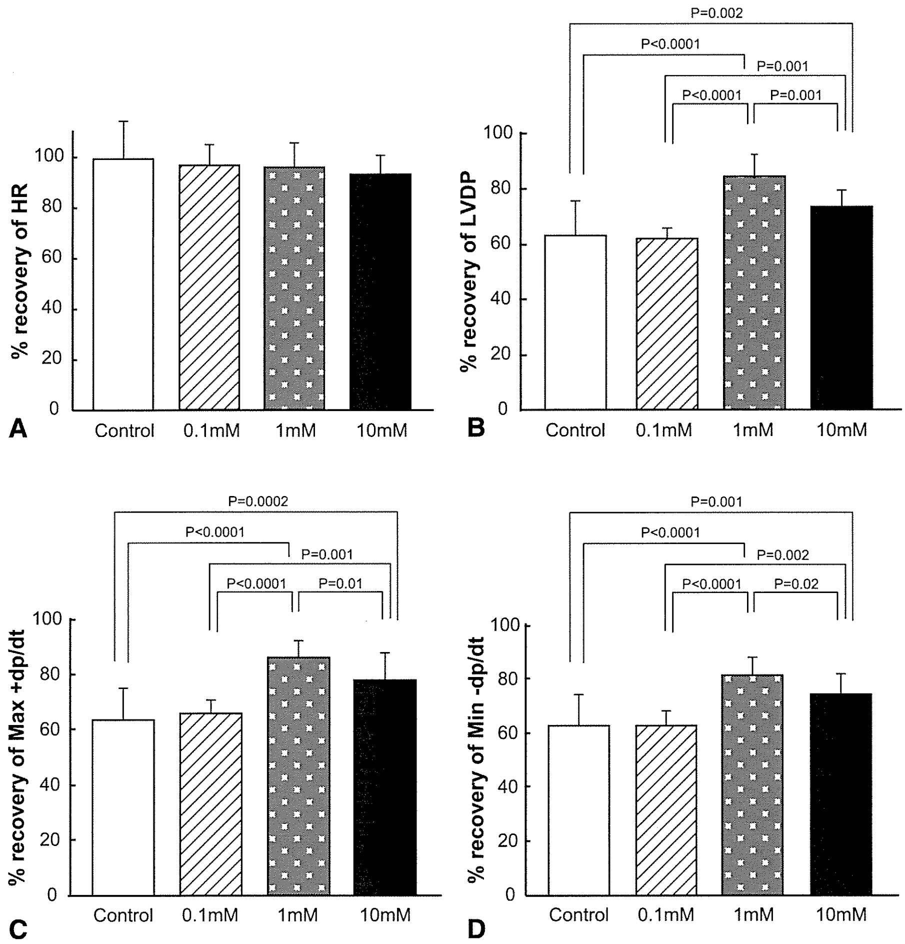

FIGURE 1. Recovery of left ventricular function at 60 minutes of reperfusion as percentage before ischemia. A, Heart rate *(HR)* did not differ significantly among groups. B–D, Recoveries of left ventricular developed pressure (*LVDP*, B), maximum positive first derivative of left ventricular pressure (*Max+dp/dt*, C), and minimum negative first derivative of left ventricular pressure (*Min−dp/dt*, D) in 1-mmol/L and 10-mmol/L groups were higher than in control and 0.1-mmol/L groups. Recoveries of left ventricular developed pressure and maximum positive and minimum negative first derivatives of left ventricular pressure were higher in 1-mmol/L group than in 10-mmol/L group. All values shown as mean ± SD, n = 12 per group.

a high dose of EGCG did not dramatically improve cardiac function after ischemia–reperfusion.

EGCG and Antioxidative Capacity

This novel pretreatment with EGCG, putting the whole body into an antioxidation state before IRI, may serve as a quite reasonable preconditioning method. One of the major theories, supported by most experimental evidence, suggests that reactive oxygen species generation is responsible for postischemic contractile dysfunction.[17] Furthermore, clinical studies have shown that reactive oxygen species generation starts before cardiac surgery and that the availabilities of protective antioxidants depend on preoperative plasma antioxidant status.[18] In this experiment, oral pretreatment with EGCG for 2 weeks before the Langendorff study had no effects on the animals' baseline cardiac parameters. Under IRI conditions, however, LV function was better maintained in the group pretreated with 1 mmol/L EGCG. The potent antioxidative capacity of green tea polyphenols could contribute to LV function recovery after reperfusion, as shown by the lower 8-OHdG indices in the 1-mmol/L and 10-mmol/L groups.

EGCG and Antiapoptotic Effects

Another mechanism possibly underlying the cardioprotective effects of EGCG is its antiapoptotic effect, exerted through oral pretreatment, which may be associated with the preservation of LV function. Oxidative stress is now considered to be a major contributor, serving as a trigger, to myocardial apoptosis. Oxidative stress–induced apoptosis and its prevention by antioxidants have therefore also been analyzed.[19] With respect to catechins, several in vitro investigations have shown EGCG to modulate multiple signal transduction pathways of apoptosis, such as mitogen-activated protein kinases (MAPKs). Furthermore, several studies have found caspase-3 cleavage, a downstream