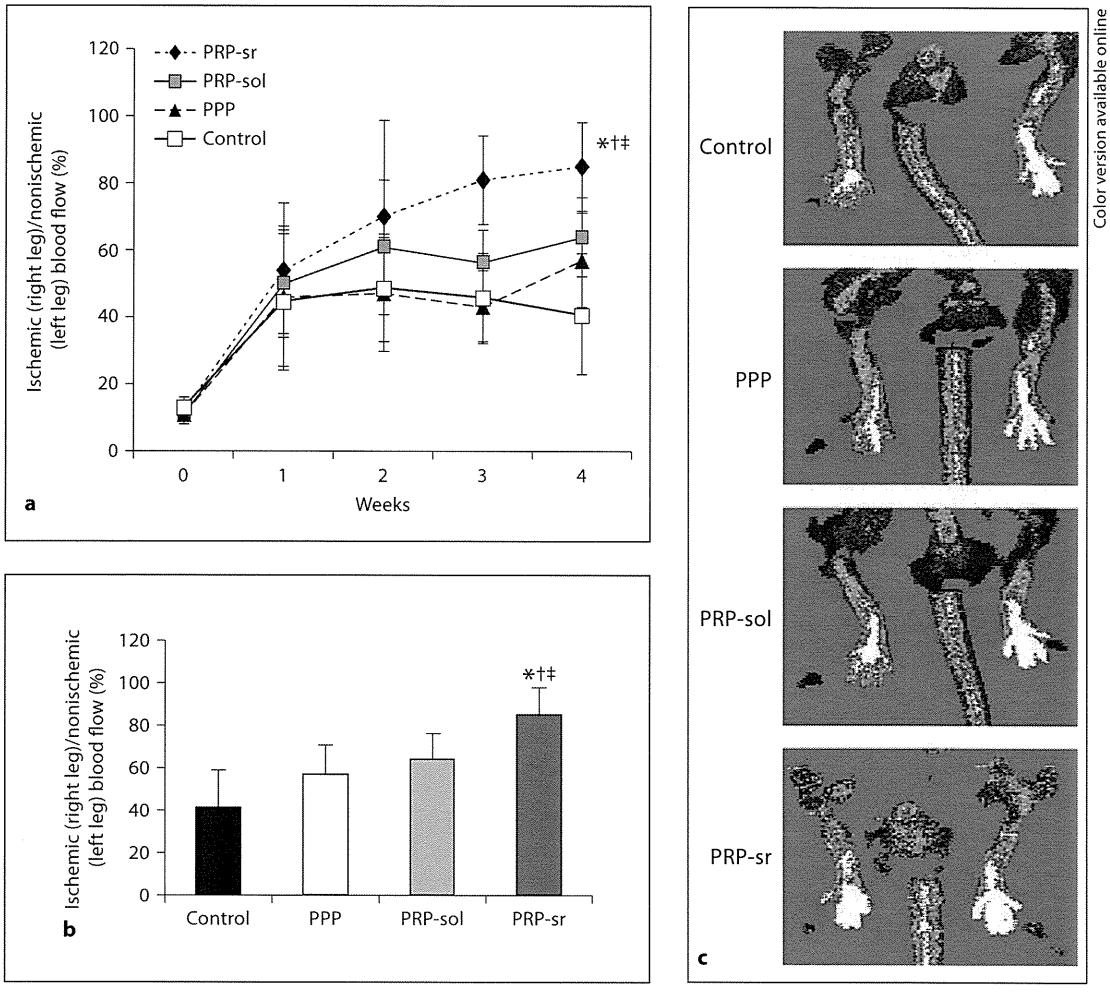
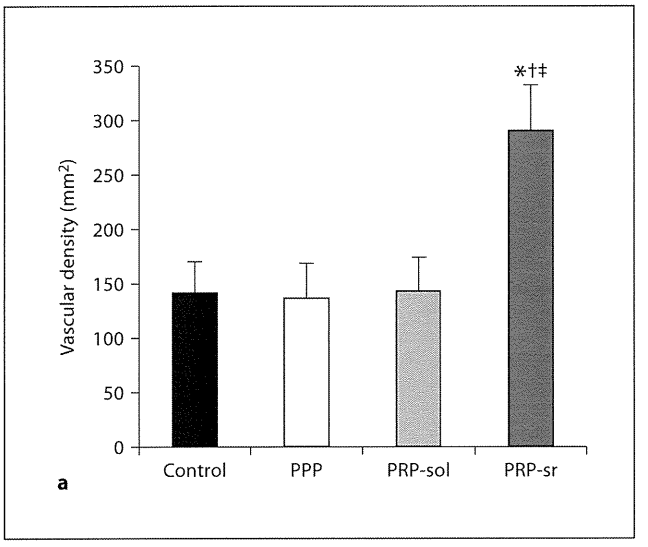


Fig. 4. a Time course of the ischemic (right leg)/nonischemic (left leg) blood perfusion ratio (%) in the different groups of mice with various treatments. **b** Ischemic/nonischemic blood perfusion ratio in the different groups at 4 weeks after various treatments. **c** LDPI in the different groups. Control group = Gelatin; PPP group = treatment with 100 μl PPP; PRP-sol group = treatment with the solution form of 100 μl PRP; PRP-sr group = treatment with the sustained-release form of 100 μl PRP; n = 10 in each group. * $p < 0.05$ vs. control group; † $p < 0.05$ vs. PPP group; ‡ $p < 0.05$ vs. PRP-sol group.

lized in the hydrogel through physicochemical interaction with gelatin molecules. The immobilized growth factors are released from the hydrogel as a result of hydrogel degradation [11, 17]. Therefore, we used gelatin hydrogel as a carrier for the growth factors in PRP.

Diabetes impairs neovascularization processes such as angiogenesis, arteriogenesis, and vasculogenesis [6–10]. In contrast, the successful reperfusion of ischemic tissue depends on complex events of neovascularization which require interplay between cells and angiogenic growth factors. For example, endothelial cells, whether preexisting or progenitor, smooth muscle cells, and pericytes are required to form complete stable vessels. VEGF causes endothelial cell proliferation and migration which result in capillary sprouting or angiogenesis. VEGF also promotes mural cell accumulation presumably through the release of PDGF-BB. Basic FGF and PDGF are chemoattractants used to smooth muscle cells. Those are also causes of the growth of smooth muscle cells as well as the enlargement of the vessel (formation of mature vessels or arteriogenesis). VEGF and SDF-1 recruit hematopoietic stem cells to the ischemic site from bone marrow via circulation. These stem cells produce capillary plexuses and eventually form mature vessels. All together, they cause



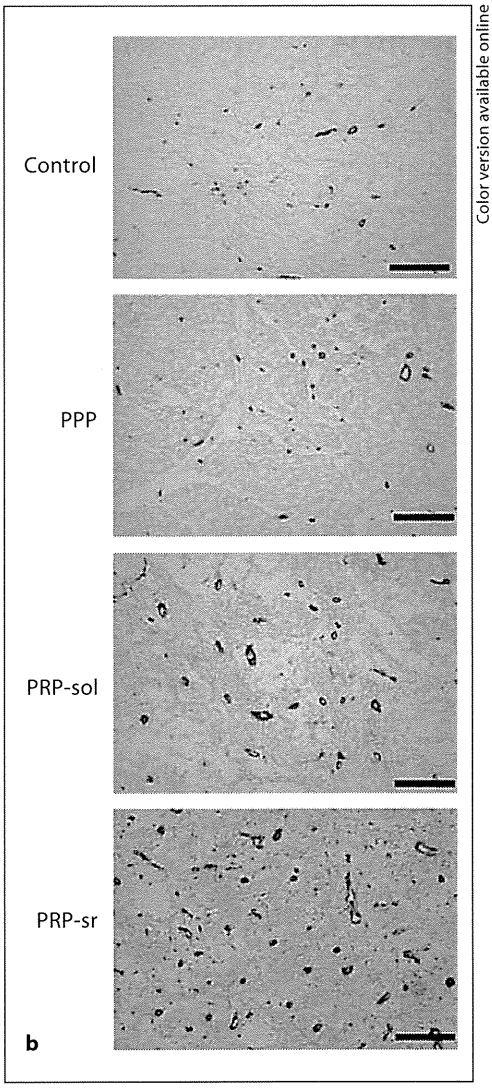


Fig. 5. a Vascular density in the ischemic calf muscles stained with anti-human vWF at 4 weeks after surgery. **b** Representative photomicrographs of the ischemic calf muscles single stained (diaminobenzene) with anti-human vWF at 4 weeks after surgery. Scale bars = 100 μm. Control group = Gelatin; PPP group = treatment with 100 μl PPP; PRP-sol group = treatment with the solution form of 100 μl PRP; PRP-sr group = treatment with the sustained-release form of 100 μl PRP; n = 10 in each group. * $p < 0.05$ vs. control group; † $p < 0.05$ vs. PPP group; ‡ $p < 0.05$ vs. PRP-sol group.

the genesis of new vessels for vascular supply in ischemic limbs [3–5]. Therefore, the coadministration of different growth factors may result in well organized neovascularization useful for therapeutic application in diabetes [4].

Langer and Gawaz [20] have suggested that platelets are important participants in tissue repair by interaction with progenitor cells. Moreover, they can exert regenerative effects themselves although they promote proinflammatory processes. This study shows that STZ-induced diabetic PRP contains large amounts of different growth factors which cause endothelial cell proliferation and capillary tube formation in vitro. Nonetheless, our previous study has shown that PRP augments ischemic neovascularization presumably due to the stimulation of angiogenesis, arteriogenesis, and vasculogenesis [11].

Diabetic Ischemia, Angiogenesis, and the Effect of PRP

VEGF is the principle stimulatory factor of angiogenesis after ischemia [21, 22], but research-based evidence has shown that bFGF, IGF-1, and SDF-1 can induce angiogenesis via direct or indirect stimulation [23–25]. Angiogenesis is impaired in diabetes due to a decrease in VEGF protein and its receptor expression, and this can be rescued by the external induction of VEGF protein [6]. Our recent and previous study showed that PRP contains a higher concentration of VEGF, bFGF, SDF-1, and IGF-1 than does PPP, which might contribute to angiogenesis in the diabetic hind limb ischemia model [11]. However, VEGF by itself promotes the formation of leaky, unstable capillaries rather than arteriogenesis, which leads us to

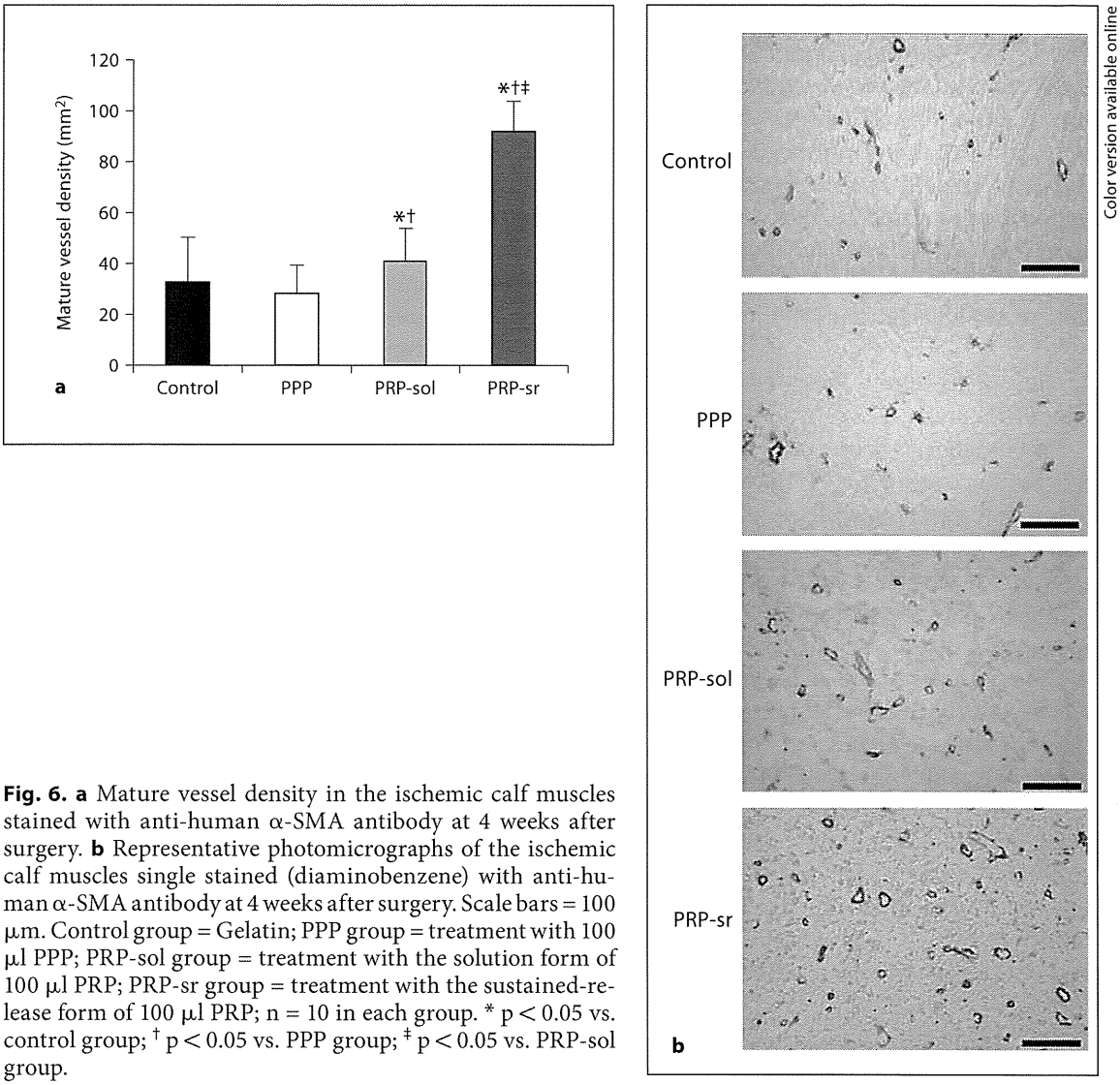


Fig. 6. a Mature vessel density in the ischemic calf muscles stained with anti-human α-SMA antibody at 4 weeks after surgery. **b** Representative photomicrographs of the ischemic calf muscles single stained (diaminobenzene) with anti-human α-SMA antibody at 4 weeks after surgery. Scale bars = 100 μm. Control group = Gelatin; PPP group = treatment with 100 μl PPP; PRP-sol group = treatment with the solution form of 100 μl PRP; PRP-sr group = treatment with the sustained-release form of 100 μl PRP; n = 10 in each group. * $p < 0.05$ vs. control group; † $p < 0.05$ vs. PPP group; ‡ $p < 0.05$ vs. PRP-sol group.

the coadministration of different growth factors for neovascularization in diabetic ischemia [4].

Diabetic Ischemia, Arteriogenesis, and the Effect of PRP

Arteriogenesis is the principle driver of the restoration of blood perfusion in the ischemic neovascularization of diabetes as well as in hypercholesterolemic models. There is strong evidence from both clinical and basic research studies, including our previous study, that arteriogenesis is severely impaired in diabetic ischemia [7–9]. Basic FGF and PDGF have strong arteriogenic effects [4, 9, 23, 26, 27]. Langer and Gawaz [20] have showed that platelets substantially recruit mesenchymal stem cells to the altered vascular wall and enhance biological functions such as proliferation, migration, and endothelial integration of mesenchymal stem cells [28]. Besides these, there is strong evidence that the expression of PDGF is impaired and there is a slight reduction of the expression of bFGF at the ischemic site of the diabetic model [29]. However, PRP contains large amounts of PDGF-BB and bFGF which could stimulate arteriogenesis in diabetic ischemia.

There is also cross talk between VEGF and bFGF and between bFGF and PDGF-BB to induce angiogenesis after ischemia [4, 29–32]. Our findings have shown that PRP effectively restores blood flow via a significant augmentation of the number of capillaries (angiogenesis) as well as mature vessels (arteriogenesis) in mouse hind

limb ischemia (fig 5, 6). Moreover, the sustained release form of PRP showed a higher effect compare to the solution form. The sustained release of PRP showed a restoration of blood flow (about 90%) within 4 weeks of treatment; while in this study we did not clarify the individual role of growth factors and signaling mechanisms of angiogenesis related to PRP in diabetes, some authors suggested that PRP acts via a concerted action of different growth factors to stimulate angiogenesis and postischemic revascularization [33].

Diabetic Ischemia, Vasculogenesis, and the Effect of PRP

Vasculogenesis is one of the predictor mechanisms of neovascularization [34]. However, there is strong evidence that vasculogenesis is impaired in diabetic ischemia [10]. It has already been reported that EPC recruitment and homing are guided by SDF-1 and VEGF. Asahara et al. [35] showed that VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. SDF-1 regulates the mobilization and local trafficking of progenitor cells to the ischemic site. De Falco et al. [36] also suggested that the transient establishment of SDF-1 favors stem cell translocation into ischemic tissue, thereby enhancing neovascularization [37]. Our data reflects that PRP contains a small amount of VEGF and a relatively large amount of SDF-1α. Massberg et al. [38] suggested that platelets secrete SDF-1α and recruit bone marrow-derived progenitor cells to the injured arterial site. Our previous study also showed that the sustained release of PRP accelerates the homing of hematopoietic progenitor cells to the ischemic site in vivo which reflects the contribution of the sustained release of PRP to vasculogenesis in hind limb ischemia [11]. Therefore, SDF-1 and VEGF in PRP might contribute to reverse the impaired vasculogenesis effect in diabetic ischemia.

Clinical Relevance

PRP is a natural, cost-effective reservoir of various growth factors which can be collected autologously. Thus, for clinical use, no special considerations concerning antibody formation and infection risk are needed. Some clinical devices to automatically prepare PRP are currently available. PRP have been consistently used in clinical settings in departments of orthopedics and plastic surgery (oral and maxillary facial) over many years [39–40]. Based on research evidence, some publications have reported positive results of PRP in either bone or soft tissue healing, yet other studies have concluded little-to-no benefit of PRP. This is likely due to the rapid degradation of growth factors in PRP since some studies suggest using sustained-release forms of PRP to achieve optimal effects. Gelatin hydrogel is used clinically as a slow, sustained release of carrier for growth factors and may be beneficial for enhancing PRP therapy.

Concluding Remarks

The sustained release of PRP using gelatin hydrogel can be a highly potent and effective modality for restoring blood perfusion to diabetic mouse hind limb ischemia. This is because the sustained release of PRP stimulates all possible aspects of impaired vascular remodeling in diabetes, such as angiogenesis, arteriogenesis and vasculogenesis, in addition to being very cost effective, autologus, and safe. These may be applicable in the clinical setting in diabetic patients with CLI.

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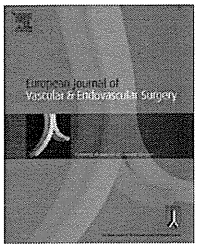

Remote Postconditioning may Attenuate Ischaemia–Reperfusion Injury in the Murine Hindlimb Through Adenosine Receptor Activation☆

H. Tsubota, A. Marui*, J. Esaki, S.C. Bir, T. Ikeda, R. Sakata

Department of Cardiovascular Surgery, Kyoto University Graduate School of Medicine Kyoto, Japan

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KEYWORDS
Remote postconditioning;
Ischaemia–reperfusion injury;
Skeletal muscle;
Adenosine

Abstract *Objective:* This study aimed to determine the effect and mechanisms of remote postconditioning (RPC) upon ischaemia–reperfusion injury (IRI) in the ischaemic mouse hindlimb.
Design: RPC is the brief application of ischaemia to remote organs immediately before reperfusion of an ischaemic target organ, and it is a novel approach to IRI attenuation.
Materials and methods: Right hindlimb ischaemia was induced in mice using a rubber tourniquet, the release of which initiated reperfusion. We established RPC by 5 min of ischaemia followed by 5 min of reperfusion in the left hindlimb immediately before right hindlimb reperfusion. The wet/dry ratio of skeletal muscle (degree of tissue oedema), myeloperoxidase (MPO) activity (accumulation of neutrophils), and nitroblue tetrazolium reduction (tissue necrosis) were evaluated. We also intra-peritoneally injected 8-sulphophenyltheophylline (SPT), an adenosine receptor inhibitor, in RPC mice.
Results: Wet/dry ratio, MPO activity and tissue necrosis were significantly lower in the RPC group than in the control group, and injection of SPT impaired the protective effect of RPC.
Conclusions: Our results show that RPC attenuated IRI in murine hindlimb ischaemia, possibly through endogenous adenosine receptor activation, and that RPC might serve as a promising therapeutic option for treating serious limb ischaemia.

Skeletal muscle ischaemia–reperfusion injury (IRI) can develop in various clinical scenarios such as during aortic, or peripheral vascular surgery or after embolic or thrombotic events and traumatic arterial injury.[1] Reperfusion injury may often be mild and self-limiting, but reperfusion of large vascular territories after prolonged ischaemia is often lethal.

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* Corresponding author. Translational Research Center, Kyoto University Hospital, 54 Kawaharacho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. Tel.: +81 75 751 3784; fax: +81 75 751 4960.
E-mail address: marui@kuhp.kyoto-u.ac.jp (A. Marui).

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Blood flow must be resumed to salvage ischaemic tissue, yet reperfusion itself paradoxically further damages ischaemic tissue.

Ischaemic pre-conditioning defined as a brief period of ischaemia followed by reperfusion that increases ischaemic tolerance to a subsequent longer ischaemic period, was first identified in the canine heart by Murry et al. in 1986,[2] and has since become established as a strategy to reduce IRI in various organ systems.[3–5] Remote pre-conditioning, which is the application of brief ischaemia to distant organs before target organ ischaemia, is also effective.[6–8] Despite this, the use of ischaemic pre-conditioning is limited by the need to apply it before unanticipated ischaemic events appear. Remote postconditioning (RPC), which is the application of brief ischaemia to distant organs after ischaemic insult and before the reperfusion of a target organ, has recently been advocated. Kerendi et al. reported that brief renal ischaemia and reperfusion applied before coronary artery reperfusion reduces the size of myocardial infarcts in rats[9] and Gritsopoulos et al. described that short-term occlusion of the carotid artery produces the same effects in rabbits.[10] These protocols allow the initiation of therapy after the onset of ischaemia, which renders RPC appropriate for unexpected ischaemic events.

Several factors might be responsible for pre-conditioning and postconditioning, including adenosine, opioid, bradykinin and ATP-sensitive K^+ channels.[11,12] Adenosine is considered an initiation trigger for pre-conditioning, as it is an important regulatory agent that exerts cytoprotective effects via the activation of G-protein-coupled receptors.[13] Liu et al. found that 8-sulphophenyltheophylline (SPT), an adenosine receptor inhibitor, reduces the protective effect of pre-conditioning in the rabbit heart.[14] However, the effects and possible mechanisms of RPC in IRI of ischaemic limbs has not been elucidated.

This study tests the hypotheses that contralateral limb ischaemia applied immediately before the onset of target limb reperfusion prevents ischaemia–reperfusion injury of the target limb and that the effects depend on the activation of adenosine receptors.

Materials and Methods

Animals

Ten-week-old male C57BL/6 mice, weighing 23–25 g, were purchased from Japan SLC (Shizuoka, Japan). The Kyoto University Animal Experiment Committee approved the experimental protocol. Animal care complied with the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council.

Induction of acute IRI in the mouse hindlimb

Mice were anesthetised with intra-peritoneal pentobarbital (60 mg kg^{-1}) and then the fur was completely shaved from both hindlimbs to facilitate measurements of limb perfusion. Body temperature was maintained at 37 °C with a heating pad during anaesthesia. As much as 30 min later, right hindlimb ischaemia was induced for 3 h using a natural rubber tourniquet (diameter, 1.5 mm; Misasa Inc., Osaka, Japan), wrapped twice around the proximal thigh, followed by 24 h of reperfusion, which was started by releasing the tourniquet all at once. The reperfusion was automatically induced with own blood flow of mice. The cessation and restoration of arterial blood flow were confirmed using a laser Doppler perfusion image (LDPI) analyser (Moor Instruments, Devon, UK). The mice remained anaesthetised throughout the ischaemic period. The mice were returned to cages and were provided with free access to food and water during reperfusion.

Establishment of RPC

RPC was established by 5 min of ischaemia followed by 5 min of reperfusion of the left hindlimb immediately before that of the right.

Experimental groups

Study 1. Investigation of the effect of RPC in skeletal muscle

The animals were randomised to the following groups: Sham (no ischaemia in bilateral legs), Control (right leg ischaemia for 3 h followed by 24 h of reperfusion) and RPC (right leg ischaemia for 3 h followed by 24 h of reperfusion accompanied by two cycles of RPC in the left hindlimb immediately before reperfusion of the right) (Fig. 1(a)).

Study 2. Investigation of the role of adenosine receptors in RPC of skeletal muscle

The adenosine receptor inhibitor, SPT, (20 mg kg^{-1}; Sigma–Aldrich, St. Louis, MO, USA) was dissolved in saline to 2.5 mg ml^{-1} immediately before use. An additional RPC group was intra-peritoneally injected with SPT 30 min before right hindlimb reperfusion (Fig. 1(b)).

Evaluation

At the end of reperfusion, the animals were euthanised with pentobarbital sodium (200 mg kg^{-1}), and the right hindlimbs were harvested. All mice were alive before the euthanisation. Sham animals were manipulated in precisely the same manner except that no rubber bands were applied (n = 8–10 per group).

Tissue oedema

The extent of skeletal muscle oedema was determined by measuring the ratio of the wet to dry tissue weight (sham, n = 8; control and RPC, n = 10). After 24 h of reperfusion, muscle samples were immediately weighed (wet weight) and then dried in an oven at 55 °C until the weight remained constant (36–48 h). Tissue oedema was detected as a relative increase in the ratio of wet to dry weight.

Myeloperoxidase (MPO) activity

Neutrophil infiltration after reperfusion injury was assessed as an increase in tissue MPO enzymatic activity (sham, n = 10; control, n = 9; RPC, n = 8). Mouse muscle samples exposed to 3 h of ischaemia followed by 24 h of reperfusion were weighed, snap-frozen in liquid nitrogen and then

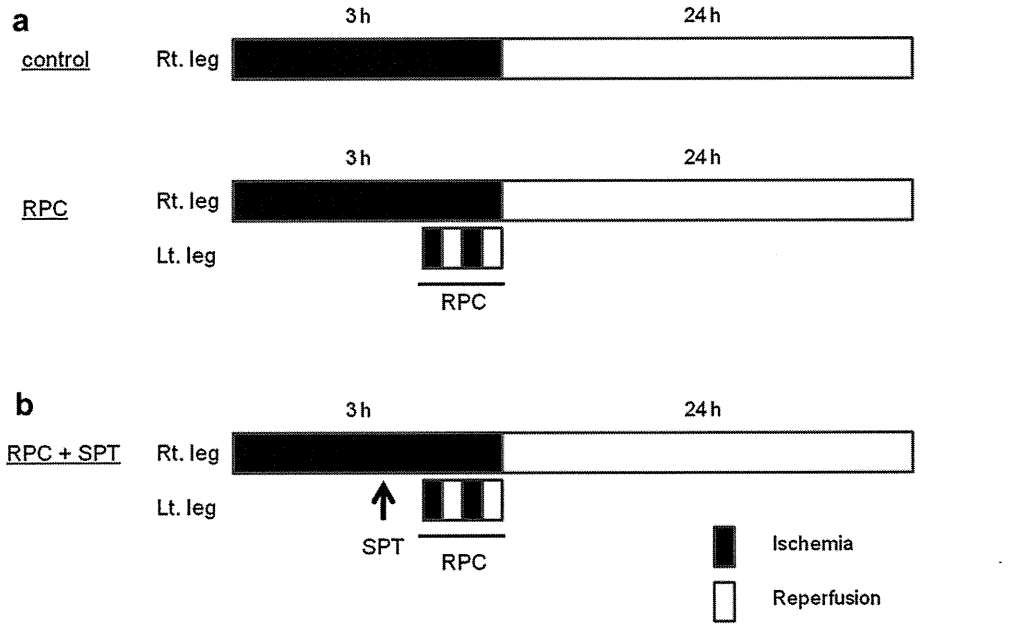

Figure 1 Experimental groups. 1. Control: Right hindlimb ischaemia for 3 h followed by 24 h of reperfusion; 2. Remote postconditioning (RPC): Right hindlimb ischaemia for 3 h followed by 24 h of reperfusion and two cycles of RPC (5 min ischaemia and 5 min reperfusion) in left hindlimb immediately before reperfusion of the right; 3. Remote postconditioning + SPT: same protocol as RPC group, but 8-sulfophenyltheophylline (SPT; 20 mg/kg) intra-peritoneally administered 30 min before starting right hindlimb reperfusion.

stored at −80 °C. Muscle tissue samples were homogenised in ice-cold potassium phosphate buffer containing 1 mM phenylmethanesulphonylfluoride (PMSF), 1 μg ml^{-1} leupeptide and 28 μg ml^{-1} aprotinine (pH 7.4) using a POLYTRON (Kinematica, Lucerne, Switzerland). The homogenates were clarified by centrifugation at 2000 rpm for 15 min at 4 °C and then, MPO activity was assayed in the supernatants using the Mouse MPO enzyme-linked immunosorbent assay (ELISA) kit (Hycult Biotechnology, Uden, the Netherlands), according to the manufacturer's instructions. Absorbance measured at 450 nm was compared with a standard curve using a microplate reader at room temperature.

Muscle necrosis

Muscle necrosis was assessed by nitroblue tetrazolium staining (sham, $n = 8$; control, $n = 9$; RPC, $n = 9$; RPC + SPT, $n = 8$). Muscle samples from mice exposed to 3 h of ischaemia followed by 24 h of reperfusion were immediately cut into three slices and incubated in 0.05% nitroblue tetrazolium for 20 min in the dark. Viable muscle stained deep blue, whereas nonviable muscle remained pale and unstained (Fig. 2). The proportion of necrosis on both sides of fixed slices was quantified using IPLab imaging software (Version 3.71 for Windows; Scanalytics Inc., Rockville, MD, USA).

Statistical analysis

All data are expressed as means ± standard deviation and as ranges. Differences between groups were assessed by an analysis of variance, followed by *post hoc* comparisons using the Bonferroni/Dunn method. All data were analysed using StatView software (Abacus Concepts, Inc., Berkeley, CA, USA).

Results

Study 1

The ratio of the wet to dry weight of muscle was lower in the RPC group than in the control group (6.0 ± 0.7 (4.9–7.1) vs. 6.7 ± 0.6 (6.0–7.5); $P = 0.007$). The degree of oedema in the control and RPC groups was higher than that of the sham group (4.0 ± 0.4 (3.6–4.7), $P < 0.001$; Fig. 3).

RPC decreased the amount of MPO activity in skeletal muscle compared with the control group (921 ± 310 U g^{-1} (398.4–1390.8) vs. 1213 ± 213 U g^{-1} (999.4–1481.3); $P = 0.008$). Levels of MPO activity were higher in both the control and RPC groups compared with the sham group (73 ± 23 U g^{-1} (55.5–133.0), $P < 0.001$; Fig. 4).

The extent of muscle necrosis in the RPC group was significantly lower than that in the control group (31.8 ± 27.5% (4.0–66.3) vs. 84.1 ± 21.0% (43.4–99.5); $P < 0.001$). The degree of muscle necrosis in both of these groups was higher than that in the sham group (1.9 ± 0.7% (0.8–2.8), $P = 0.008$ and $P < 0.001$, respectively; Fig. 5).

Study 2

RPC together with the SPT injection (67.0 ± 29.5% (16.3–93.3)) abrogated the protective effect of RPC alone ($P < 0.001$; Fig. 5).

Discussion

RPC decreased tissue oedema, MPO activities and tissue necrosis of IRI in the murine hindlimb, and the adenosine

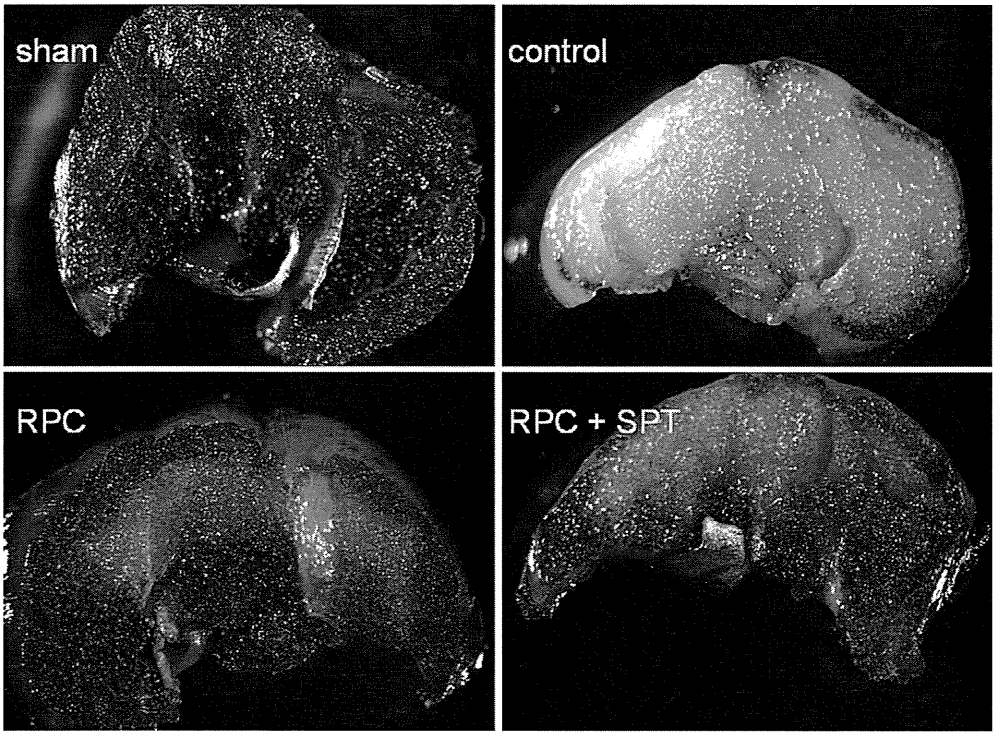

Figure 2 Gastrocnemius muscles stained with nitroblue tetrazolium. Viable muscle is obviously stained, and non-viable muscle is pale and unstained.

receptor inhibitor, SPT, reduced the decrease in tissue necrosis caused by RPC. These results indicate that RPC reduces IRI damage to the murine hindlimb through an adenosine-dependent mechanism.

Among several suggested protocols for RPC, the major distinguishing feature is whether distant organ ischaemia and reperfusion are applied before or after reperfusion of a target organ. Kerendi et al. reported that brief renal ischaemia and reperfusion applied just before coronary artery reperfusion reduces the size of myocardial infarcts in rats.[9] On the other hand, Gritsopoulos et al. described that short-lived occlusion of the carotid artery after reperfusion of the coronary artery decreases the size of myocardial infarcts in rabbits.[10] Both reports describe these methods as 'remote postconditioning'. Furthermore, Eberlin et al. demonstrated sequential ischaemia in the contralateral limb as 'remote postconditioning' of murine skeletal muscle.[15] A total of 2 h of contralateral limb ischaemia following 20 min of reperfusion after initial limb ischaemia protected the initially ischaemic limb, whereas injury to the secondarily ischaemic limb was severe. Therefore, this protocol requires further consideration before it can be clinically applied.

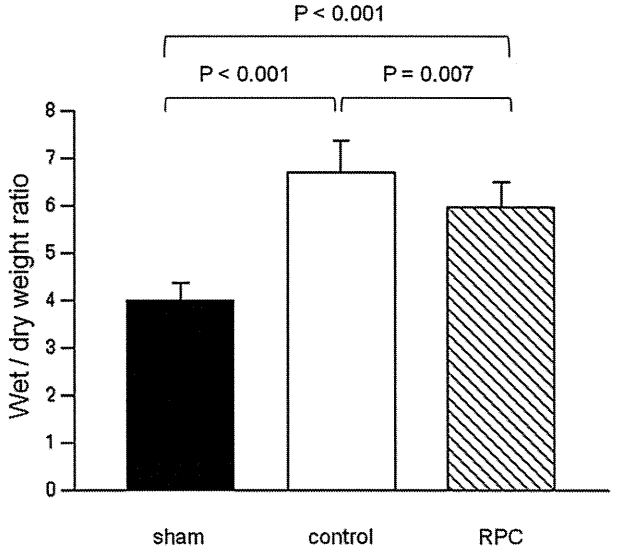

Figure 3 Ratio of wet to dry muscle weight after 3 h of ischaemia and 24 h of reperfusion in murine hindlimb IRI. Data are means ± SD; sham, $n = 8$; control and RPC, $n = 10$.

Here, we produced ischaemia and reperfusion in the opposite hindlimb immediately before reperfusion of the target limb. We tested a protocol comprising two cycles of 5 min of ischaemia and 5 min of reperfusion. The degree of necrosis after RPC with one such cycle was slightly reduced compared with the control group (57.7 ± 33.7% vs. 84.1 ± 21.0%; $P = 0.008$), whereas two cycles significantly reduced muscle damage. Further investigation is required to identify the optimal protocol with due regard to the importance of urgent reperfusion of ischaemic limbs, and to preventing iatrogenic damage to the contralateral limb.

RPC confers three clinical advantages. First, it can be applied after the onset of sudden ischaemic events. The protective effects of ischaemic pre-conditioning and ischaemic remote pre-conditioning have been elucidated experimentally; however, they have limited application (elective vascular surgery, for example) given the need to treat before ischaemia. Because the period before perfusion is resumed after sudden arterial thrombosis and vascular trauma lasts

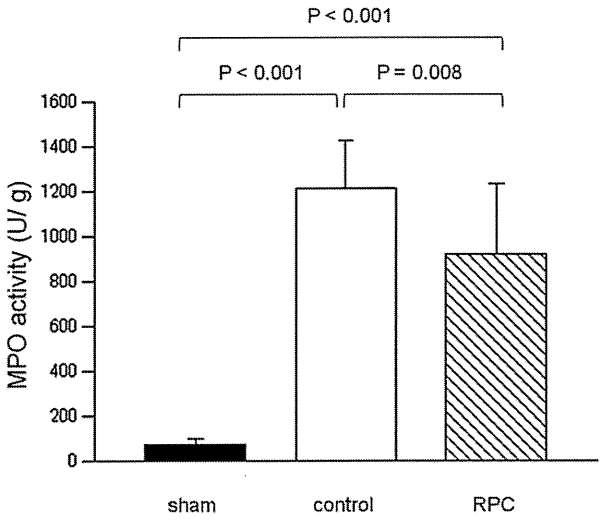

Figure 4 Muscle neutrophilic myeloperoxidase activity after 3 h of ischaemia and 24 h of reperfusion in murine hindlimb IRI. Data are means ± SD; sham, $n = 10$; control, $n = 9$; RPC, $n = 8$.

much longer than that after elective vascular surgery, RPC might become an effective adjuvant therapy for these problems. Second, upper limb ischaemia can be simply induced with low risk by inflating a blood pressure cuff. Intervention in a diseased artery is undesirable, because of the risk of inducing iatrogenic thrombosis and vascular injury. Arteriosclerosis is generally milder in upper, than lower limbs; hence, brief compression of brachial artery is acceptable. Third, this procedure could be cost-effective because it can be achieved with routinely available equipment, and unique drugs are not required.

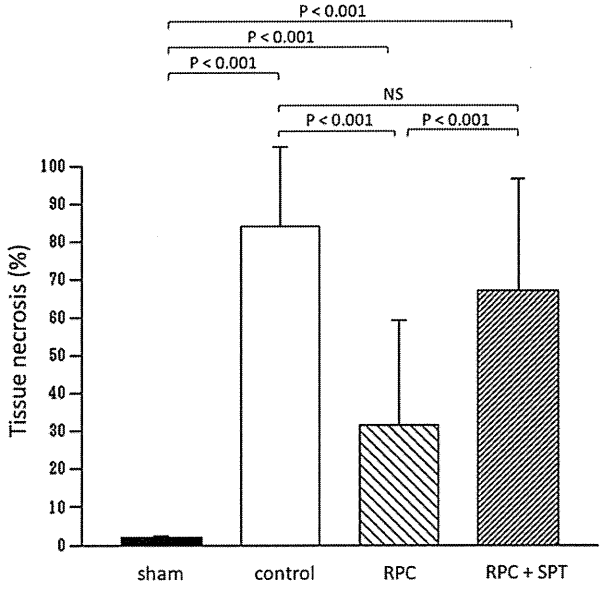

Figure 5 Muscle necrosis after 3 h of ischaemia and 24 h of reperfusion in murine hindlimb IRI. Adenosine receptor inhibitor, 8-sulfophenyltheophylline (SPT, 20 mg/kg), was intraperitoneally injected at 30 min before right hindlimb reperfusion. Data are means ± SD; sham, $n = 8$; control, $n = 9$; RPC, $n = 9$; RPC + SPT, $n = 8$.

Several investigators have suggested that adenosine receptor activation is involved in the protective effects of ischaemic pre-conditioning. Ischaemic tissue rapidly degrades adenosine triphosphate (ATP) to adenosine, which subsequently activates protein kinase C via phospholipases. These events lead the activation of mitochondrial ATP-sensitive K^+ channels that might be the end effectors.[16]

Adenosine is also considered to play an important role in mediating the protective effect of remote pre-conditioning. Three putative mechanistic pathways have been proposed to explain how remote organs are protected.[17] The humoral theory suggests that adenosine released into the bloodstream by ischaemic tissue binds to receptors in remote organs, which in turn triggers the intracellular pathway that mediates protection.

The neural theory suggests that adenosine released by ischaemic tissue activates local afferent nerves, which in turn activates efferent nerves to protect remote organs.[18,19]

The inflammatory suppression theory suggests that adenosine released from ischaemic tissue suppresses the systemic inflammatory response.[20,21] Because neutrophils are believed to play an important role in IRI, we assessed neutrophil infiltration as an increase in tissue MPO activity. We found that RPC reduced MPO activity after 24 h of reperfusion. This indicates that RPC attenuated neutrophil accumulation, which might represent one of the mechanisms through which RPC conferred a protective effect against IRI.

Due to the dearth of information about RPC, little has been reported about its mechanism of action. We found here that the non-selective adenosine receptor antagonist, SPT, suppressed the reduction in the degree of necrosis induced by RPC, which makes the adenosine-mediated mechanism convincing. Our results empirically showed that RPC protects skeletal muscle through adenosine-dependent mechanisms.

The present study has several limitations. We used young healthy mice, although ischaemic events tend to occur in the aged and diseased populations. The effects of ischaemic conditioning might be negated or inhibited by ageing, disease states such as diabetes or hyperlipidaemia and drugs that are commonly administered to such patients. These factors might explain the unsatisfactory results of clinical trials of ischaemic conditioning.[22] We used pentobarbital for anaesthesia, which might affect the microcirculation, and we did not use heparin, but confirmed that the blood flow in both limbs was similar before and after ischaemia using an LDPI analyser. However, we have to consider thrombosis in a contralateral limb induced by RPC in the clinical environment. Because we did not construct dose–response curves for SPT, we cannot provide data about other doses. Further studies are required to address the above limitations.

In conclusion, RPC applied just before reperfusion attenuates damage caused by IRI in the murine hindlimb, and adenosine seems to be involved in this process. Further investigations are required to clarify the optimal protocol and define the mechanistic pathways of RPC.

Conflict of Interest/Funding

None.

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ORIGINAL ARTICLE

Significance of off-pump coronary artery bypass grafting compared with percutaneous coronary intervention: a propensity score analysis

Akira Marui[a,b,*], Takeshi Kimura[c], Shiro Tanaka[b], Yutaka Furukawa[d], Toru Kita[d], Ryuzo Sakata[a], and the CREDO-Kyoto Investigators

[a] Department of Cardiovascular Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan
[b] Translational Research Center, Kyoto University Hospital, Kyoto, Japan
[c] Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan
[d] Kobe City Medical Center General Hospital, Kobe, Japan

* Corresponding author. Tel.: +81-75-7513784; fax: +81-75-7514960; e-mail: marui@kuhp.kyoto-u.ac.jp (A. Marui).

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Abstract

OBJECTIVE: Although there have been several studies that compared the efficacy of percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG), the impact of off-pump CABG (OPCAB) has not been well elucidated. The objective of the present study was to compare the outcomes after PCI, on-pump CABG (ONCAB), and OPCAB in patients with multivessel and/or left main disease.

METHODS: Among the 9877 patients undergoing first PCI using bare-metal stents or CABG who were enrolled in the CREDO-Kyoto Registry, 6327 patients with multivessel and/or left main disease were enrolled into the present study (67.9 ± 9.8 years old). Among them, 3877 patients received PCI, 1388 ONCAB, and 1069 OPCAB. Median follow-up was 3.5 years.

RESULTS: Comparing PCI with all CABG (ONCAB and OPCAB), propensity-score-adjusted all-cause mortality after PCI was higher than that CABG (hazard ratio (95% confidence interval): 1.37 (1.15–1.63), $p < 0.01$). The incidence of stroke was lower after PCI than that after CABG (0.75 (0.59–0.96), $p = 0.02$). CABG was associated with better survival outcomes than PCI in the elderly (interaction $p = 0.04$). Comparing OPCAB with PCI or ONCAB, propensity-score-adjusted all-cause mortality after PCI was higher than that after OPCAB (1.50 (1.20–1.86), $p < 0.01$). Adjusted mortality was similar between ONCAB and OPCAB (1.18 (0.93–1.51), $p = 0.33$). The incidence of stroke after OPCAB was similar to that after PCI (0.98 (0.71–1.34), $p > 0.99$), but incidence of stroke after ONCAB was higher than that after OPCAB (1.59 (1.16–2.18), $p < 0.01$).

CONCLUSIONS: In patients with multivessel and/or left main disease, CABG, particularly OPCAB, is associated with better survival outcomes than PCI using bare-metal stents. Survival outcomes are similar between ONCAB and OPCAB.

Keywords: Coronary artery bypass grafting • Percutaneous coronary intervention • Off-pump

INTRODUCTION

Several randomized controlled trials (RCTs) and meta-analyses comparing percutaneous coronary interventions (PCIs) with coronary artery bypass grafting (CABG) demonstrated similar long-term survival outcomes for PCI and CABG [1–4]. However, these studies may not accurately reflect current clinical practice of coronary revascularization for following reasons. First, these studies had limitations that mitigated against the prognostic and symptomatic benefits of CABG in many patients with left main disease and/or more complex disease in 'real-world' clinical practice [5,6]. Second, technical development of CABG has not been well reflected in those studies. CABG was primarily performed with the use of cardiopulmonary bypass (on-pump CABG (ONCAB)). In the mid-1990s, CABG without cardiopulmonary bypass (off-pump CABG (OPCAB)) has been introduced to reduce postoperative complications such as stroke which are associated with the use of cardiopulmonary bypass [7,8]. Thus, it is important to investigate the impact of OPCAB in patients with more complex coronary lesions.

The Coronary REvascularization Demonstrating Outcome Study in Kyoto (CREDO-Kyoto) is a multicenter registry in Japan enrolling consecutive 9877 patients undergoing first PCI or CABG and excluding those patients with acute myocardial infarction within a week before index procedure [9]. We reported that adjusted survival outcomes tended to be better after CABG than those after PCI in patients with multivessel disease without left main disease (hazard ratio (HR), 95% confidence interval (CI): 1.23 (0.99–1.53), $p = 0.06$ for PCI vs CABG). However, we did not evaluate the impact of OPCAB on outcomes. Thus, the purpose of the present study was to compare the outcomes of PCI, ONCAB, or OPCAB using the data from the CREDO-Kyoto Registry by propensity score model. To reflect the real world of coronary revascularization in the analysis, we included patients with multivessel and/or left main disease.

PATIENTS AND METHODS

Study population

The CREDO-Kyoto is a multicenter registry in Japan enrolling consecutive patients undergoing first PCI or CABG and excluding those patients with acute myocardial infarction within a week before index procedure. This study was approved by the institutional review boards or ethics committees of all participating institutions. As the study subjects were retrospectively 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 the follow-up [9].

Between January 2000 and December 2002, 9877 patients were identified to have undergone either CABG (2999 patients) or PCI (6878 patients) without prior history of coronary revascularization. Among them, patients with multivessel and/or left main coronary artery disease were included in the present study. Four hundred eighty-four patients undergoing concomitant valvular, left ventricular, or major vascular operation were excluded from the current analysis. Patients with single-vessel disease without left main disease (PCI: 3001 patients and CABG: 65 patients) were also excluded. Therefore, the study group comprised 6327 patients with multivessel and/or left main coronary artery disease undergoing first coronary revascularization (PCI: 3877 patients and CABG: 2450 patients).

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. If sufficient follow-up data are unavailable, the investigators contact patients by telephone or letter. If the patient died at the time of contact, the investigators try to obtain data from the family regarding death including non-fatal events before the time of death as great an extent as possible.

Baseline clinical characteristics, such as myocardial infarction, heart failure, diabetes, hypertension, current smoker status, atrial fibrillation, chronic obstructive lung disease, and malignancy, were regarded as present when these diagnoses were recorded in the hospital charts. 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 Cockcroft–Gault formula was less than 60 ml min^{-1}. Anemia was defined as blood hemoglobin level <12 g dl^{-1} as previously described [9].

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 [1]. Within 1 week of the index procedure, only Q-wave myocardial infarction was adjudicated as myocardial infarction. Stroke was defined as any new permanent global or focal neurologic deficit that could not be attributed to other neurologic or medical processes. In the majority of patients, strokes were diagnosed by neurologists and confirmed by computed tomography or magnetic resonance imaging head scans. Stroke at follow-up was defined as symptomatic stroke.

Primary endpoint was death from any cause. Secondary endpoints were cardiovascular death, stroke, myocardial infarction, composite cardiovascular event (cardiovascular death, stoke, or myocardial infarction), and need for any revascularization procedures (PCI or CABG) during the follow-up period.

Statistical analyses

All continuous variables are expressed as the mean ± standard deviation. Differences in baseline characteristics across the three groups were examined by analysis of variance of χ^2-test.

Propensity scores, which were the probabilities that a patient would undergo PCI or probability that a patient would undergo OPCAB, were calculated for each patient. The propensity scores were estimated with multivariable logistic regression analyses separately. Confounding factors in the logistic regression included age, gender, body mass index, emergency procedure, prior myocardial infarction, congestive heart failure, stroke, peripheral arterial disease, atrial fibrillation, chronic obstructive pulmonary disease, malignancy, hypertension, diabetes, 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, ONCAB, or OPCAB are compared by the Cox proportional hazard models stratified by the quartiles of propensity scores. Propensity-score-adjusted HRs, 95% CIs, and p values are reported. The p values for multiple comparisons, namely PCI versus OPCAB and ONCAB versus OPCAB, were adjusted by the Bonferroni correction, that is, we multiplied the original p values by 2. All reported p values were two sided. Subgroup analysis was also conducted with regard to five prespecified risk factors, including triple-vessel disease, diabetes, left ventricular dysfunction, proximal LAD disease, and the elderly [9], and p values for the interaction term were reported additionally.

All reported p values were two sided. All analyses were conducted by a statistician with the use of SAS software version 9.2 (SAS Institute Inc. North Carolina, USA) and S-Plus version 7.0 (Insightful Corp. Seattle, USA). The authors had full access to the data and take responsibility for their integrity. All authors have read and agreed to the manuscript as written.

RESULTS

Baseline characteristics

Among the 6327 patients with multivessel and/or left main disease, 3877 patients (61%) received PCI, 1381 ONCAB (22%), and 1069 OPCAB (17%). Baseline characteristics of the patients in the three groups are shown in Table 1. ONCAB and OPCAB groups generally included more high-risk patients, such as those

Table 1: Baseline characteristics

	PCI (n = 3877)		ONCAB (n = 1381)		OPCAB (n = 1069)		p value*
Age	68.3 ± 10.0		66.3 ± 9.3		68.6 ± 9.4		<0.01
Male gender	2704	70%	1000	72%	757	71%	0.17
Body mass index	23.7 ± 3.3		23.5 ± 3.2		23.6 ± 3.2		0.02
No. of diseased vessels	2.36 ± 0.53		2.58 ± 0.73		2.55 ± 0.74		<0.01
Two-vessel disease	2351	61%	305	22%	271	25%	<0.01
Triple-vessel disease	1461	38%	958	69%	707	66%	<0.01
Left main disease	165	4%	410	30%	332	31%	<0.01
Proximal LAD disease	1545	40%	791	57%	639	60%	<0.01
Total occlusion	1301	34%	672	49%	457	43%	<0.01
Emergency procedure	191	5%	77	6%	75	7%	0.03
Ejection fraction (%)	62.1 ± 13.6		58.6 ± 15.0		61.2 ± 13.7		<0.01
Prior myocardial infarction	1006	26%	489	35%	342	32%	<0.01
Heart failure	569	15%	316	23%	303	28%	<0.01
Atrial fibrillation	254	7%	80	6%	60	6%	0.40
History of stroke	607	16%	237	17%	289	27%	<0.01
Peripheral artery disease	367	9%	239	17%	243	23%	<0.01
Chronic pulmonary disease	83	2%	30	2%	22	2%	0.98
Current smoker	1056	27%	355	26%	250	23%	0.04
Malignancy	321	8%	80	6%	79	7%	0.01
Diabetes	1651	43%	642	46%	499	47%	0.01
Hypertension	2810	72%	918	66%	805	75%	<0.01
Hyperlipidemia	1955	50%	710	51%	609	57%	0.00
Chronic kidney disease	1411	36%	532	39%	426	40%	0.08
Hemodialysis	167	4%	69	5%	54	5%	0.42
Hemoglobin (g dr^{1})	13.1 ± 2.0		12.7 ± 2.0		12.6 ± 2.0		<0.01
Medications at discharge							
Statins	1287	33%	207	15%	289	27%	<0.01
Aspirin	3441	89%	1080	78%	957	90%	<0.01
Thienopyridines	2964	76%	87	6%	197	18%	<0.01
ACE inhibitor	1025	26%	135	10%	136	13%	<0.01
ARB	599	15%	102	7%	153	14%	<0.01
β antagonist	847	22%	123	9%	117	11%	<0.01
Calcium antagonist	2320	60%	801	58%	682	64%	0.02
Nitrates	2805	72%	677	49%	457	43%	<0.01

Mean ± standard deviation, or number of patients and percentage. LAD: left anterior descending artery; ACE: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers.
p value is for comparison among PCI, ON- and OPCAB by analysis of variance or x^2 test.

with left ventricular dysfunction, heart failure, prior myocardial infarction, chronic kidney disease, history of stroke, and anemia. Patient with diabetes was more common in ONCAB and OPCAB. Regarding the complexity of coronary artery anatomy, ONCAB and OPCAB groups included more complex patients, such as those with triple-vessel disease, left main disease, involvement of proximal LAD, and total occlusion. In the PCI group, bare-metal stents were used in 85% of patients. None of the patients received drug-eluting stents. Medications such as statins, thienopyridines, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta blockers, and nitrates were more frequently used in the PCI group than in the CABG group. Types of bypass grafts are shown in Table 2. OPCAB was performed using more arterial grafts than ONCAB.

Table 2: CABG data

	ONCAB (n = 1381)		OPCAB (n = 1069)		p value
No. of anastomotic sites	3.3 ± 1.0		3.2 ± 1.2		<0.01
Type of bypass grafts					
Left internal thoracic artery	1263	91%	1000	94%	0.05
Right internal thoracic artery	185	13%	577	54%	<0.01
Right gastroepiploic artery	279	20%	371	35%	<0.01
Radial artery	550	40%	253	24%	<0.01
Saphenous vein	1035	75%	462	43%	<0.01
Total arterial revascularization	346	25%	607	57%	<0.01

Mean ± standard deviation, or number of patients and percentage.

PCI versus CABG

Clinical follow-up were completed in 98% at 1 year and 95% at 2 years. The median follow-up period was 1314 days in the PCI group (interquartile range, 979–1649) and 1267 days in the CABG group (interquartile range, 950–1584).

Propensity score analysis showed that all-cause mortality adjusted for confounders was higher after PCI than that after CABG (HR (95% CI): 1.37 (1.15–1.63), p < 0.01, Table 3). This finding was similar when patients were stratified to propensity score and institutes (1.30 (1.06–1.61), p = 0.01). The incidences

Table 3: Hazard ratios for outcomes after PCI compared with that after CABG adjusted by propensity score stratification

	Number of events		HR	95% CI	*p* value
	PCI (*n* = 3877)	CABG (*n* = 2450)			
All-cause death	454	279	1.37	1.15–1.63	<0.01
Cardiovascular death	282	186	1.39	1.12–1.73	<0.01
Stroke	192	171	0.75	0.59–0.96	0.02
Myocardial infarction	188	83	1.82	1.34–2.47	<0.01
Composite event[a]	564	369	1.19	1.02–1.39	0.03
Any revascularization	1873	277	6.72	5.84–7.73	<0.01

[a] Composite event: cardiovascular death, stroke, or myocardial infarction e.g. all-cause mortality after PCI was 1.37 times higher than that after CABG ($p < 0.01$), whereas stroke rate after PCI was 0.75 times lower than CABG ($p = 0.02$).

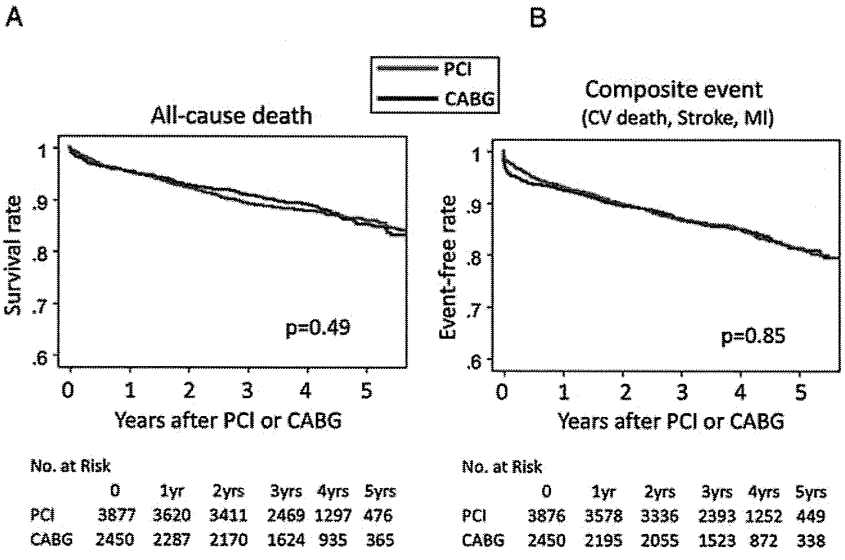

Figure 1: Kaplan–Meier curves for each endpoint comparing PCI with CABG. CV: cardiovascular; MI: myocardial infarction.

after PCI were higher than those after CABG in the adjusted analysis regarding cardiovascular death (1.39 (1.12–1.73), $p < 0.01$) and myocardial infarction (1.82 (1.34–2.47), $p < 0.01$). However, the incidence of stroke was lower after PCI (0.75 (0.59–0.96), $p = 0.02$). The incidence of composite cardiovascular event was higher after PCI (1.19 (1.02–1.39), $p = 0.03$). The incidence of repeated revascularization was far higher after PCI (6.72 (5.84–7.73), $p < 0.01$). Kaplan–Meier survival curve and event-free curve for composite cardiovascular event are presented in Fig. 1A and B.

A forest plot in Fig. 2 presents subset analysis for all-cause death after adjusted for propensity score. Interaction *p* value indicated that CABG was associated with better survival outcomes than PCI particularly in patients with the age of ≥75 (interaction $p = 0.04$) and possibly in patients with LVEF of <40% ($p = 0.09$).

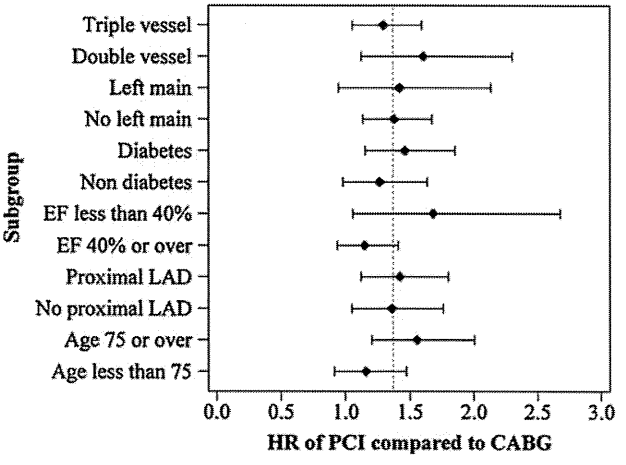

Figure 2: Forest plot of propensity-score-adjusted hazard ratios for death after PCI as compared with that after CABG in subgroups. Dashed line indicates hazard ratio in all patients of 1.37. Interaction tests, which are design to detect whether the specific factor modifies the effect of PCI relative to CABG, were significant for age ($p = 0.04$) and borderline for ejection fraction ($p = 0.09$). These indicate that CABG is associated with better survival outcomes than PCI particularly in patients with the age of ≥75 and possibly in patients with LVEF of <40%.The other interaction tests were not significant.

OPCAB versus PCI or ONCAB

Propensity score analysis showed that all-cause mortality after PCI was higher than that after OPCAB (1.50 (1.20–1.86), $p < 0.01$; Table 4), but similar between ONCAB and OPCAB

Table 4: Hazard ratios for outcomes after PCI or ONCAB compared with that after OPCAB adjusted by propensity score stratification

	Number of events			Versus OPCAB	HR	95% CI	*p* value*
	PCI (*n* = 3877)	ONCAB (*n* = 1381)	OPCAB (*n* = 1069)				
All-cause death	454	154	125	PCI	1.50	1.20–1.86	<0.01
				ONCAB	1.18	0.93–1.51	0.33
Cardiovascular death	282	113	73	PCI	1.74	1.32–2.31	<0.01
				ONCAB	1.49	1.11–2.02	0.02
Stroke	192	107	64	PCI	0.98	0.71–1.34	1.00
				ONCAB	1.59	1.16–2.18	<0.01
Myocardial infarction	188	54	29	PCI	2.41	1.57–3.71	<0.01
				ONCAB	1.61	1.01–2.55	0.09
Composite event[a]	564	230	139	PCI	1.52	1.24–1.86	<0.01
				ONCAB	1.53	1.24–1.90	<0.01
Any revascularization	1873	152	125	PCI	6.61	5.46–8.01	<0.01
				ONCAB	0.97	0.77–1.24	1.00

[a] Composite event : cardiovascular death, stroke, or myocardial infarction.
*Adjusted for multiple comparison by the Bonferroni correction, i.e. we multiplied the original *p* values by 2 e.g. all-cause mortality after PCI was 1.50 times higher than that after OPCAB ($p < 0.01$), whereas that after ONCAB was similar to OPCAB (hazard ratio = 1.18, $p = 0.33$).

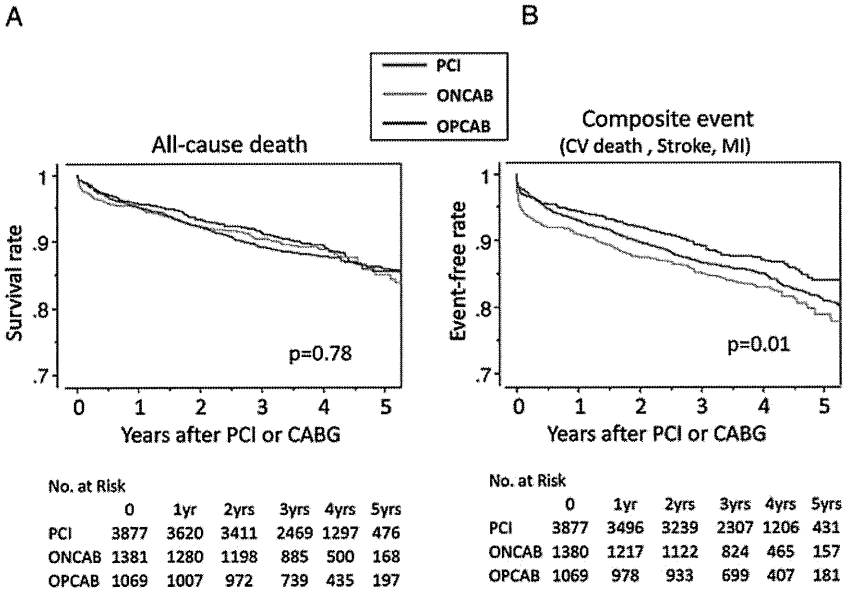

Figure 3: Kaplan–Meier curves for each endpoint comparing PCI, ONCAB, and OPCAB. CV: cardiovascular; MI: myocardial infarction.

(1.18 (0.93–1.51), $p = 0.33$). Cardiovascular mortality after PCI and ONCAB was higher than that after OPCAB (1.74 (1.32–2.31), $p < 0.01$ and 1.49 (1.11–2.02), $p = 0.02$, respectively). The incidence of stroke after OPCAB was similar to that after PCI (0.98 (0.71–1.34), $p > 0.99$), but incidence of stroke after ONCAB was higher than that after OPCAB (1.59 (1.16–2.18), $p < 0.01$). The incidence of myocardial infarction after PCI was higher than that after OPCAB (2.41 (1.57–3.71), $p < 0.01$). The incidence of composite cardiovascular event after OPCAB was lower than that after PCI (1.52 (1.24–1.86), $p < 0.01$) or ONCAB (1.53 (1.24–1.90), $p < 0.01$). These findings were similar when patients were stratified to propensity score and institutes. Kaplan–Meier survival curve and event-free curve for composite cardiovascular event are presented in Fig. 3A and B.

Forest plots in Fig. 4 show subset analysis for comparison of all-cause mortalities after OPCAB, ONCAB, and PCI. There were no significant interactions between PCI compared to OPCAB or ONCAB compared to OPCAB, and subgroups, indicating that there was no evidence against consistency of the adjusted HRs across subgroups.

DISCUSSION

Main findings

In the present study, we investigated the impact of CABG, particularly OPCAB, on long-term outcomes after PCI or CABG in Japanese patients with multivessel and/or left main disease. In this

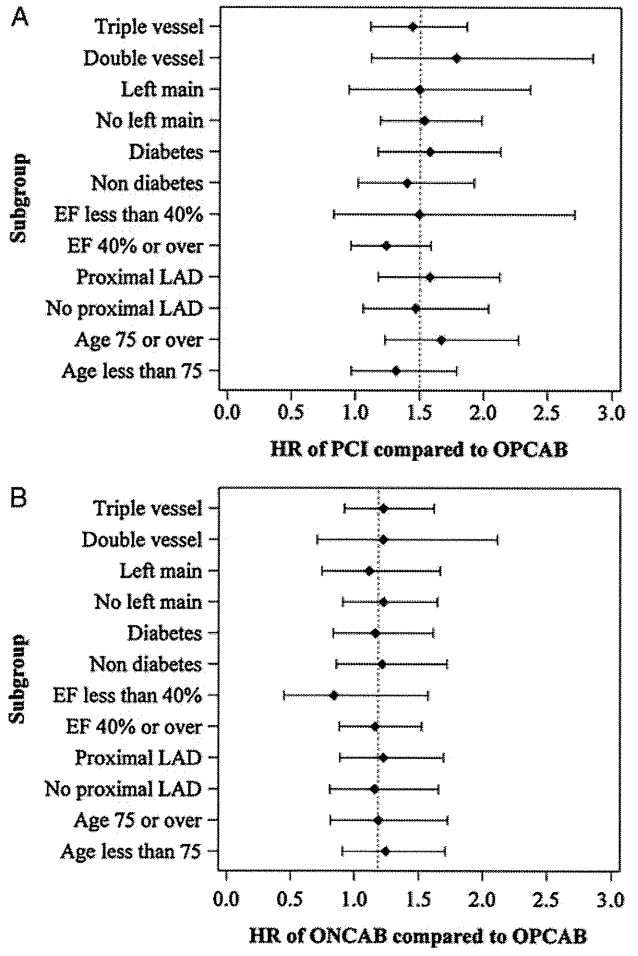

Figure 4: Forest plot of propensity-score-adjusted hazard ratios for death after PCI (A) or ONCAB (B) as compared with that after OPCAB in subgroups. Dashed line indicates hazard ratio in all patients of 1.50 (A) or 1.18 (B). Interaction tests, which are design to detect whether the specific factor modifies the effect of PCI or ONCAB relative to OPCAB, were not significant for all subgroups.

population, we showed that CABG reduced the incidences of propensity adjusted all-cause and cardiovascular mortality compared with PCI and reduced the incidences of myocardial infarction and repeated revascularization. In addition, CABG was associated with better adjusted survival outcomes than PCI in high-risk subgroups such as with triple-vessel disease, diabetes, left ventricular dysfunction, proximal LAD disease, and the elderly. However, CABG was associated with higher stroke rate than PCI.

When comparing OPCAB with PCI or ONCAB, OPCAB was associated with better survival outcomes than PCI. Importantly, OPCAB significantly reduced the incidence of stroke compared with ONCAB, which was similar to PCI. OPCAB reduced the incidence of composite cardiovascular event in comparison to PCI or ONCAB. Need for any revascularization of OPCAB was far lower than that of PCI, which was similar to ONCAB. OPCAB was associated with better adjusted survival outcomes than PCI in high-risk subgroups such as with triple-vessel disease, diabetes, proximal LAD disease, and the elderly. There were no differences in survival outcomes between ONCAB and OPCAB in those prespecified high-risk subgroups. These outcomes strongly support the novel guidelines on myocardial revascularization of European Society of Cardiology/European Association for Cardio-Thoracic Surgery (ESC/EACTS) [10], which strongly recommends CABG in complex coronary lesions such as triple-vessel and/or left main disease.

PCI versus CABG in multivessel without left main disease

A number of RCTs and meta-analyses have compared revascularization by PCI or CABG in the management of coronary artery disease with multivessel without left main disease [1–4]. A meta-analysis of four RCTs comparing PCI that involves bare-metal stents with CABG (Arterial Revascularization Therapies Study (ARTS), Coronary Angioplasty With Stenting Versus Coronary Bypass Surgery in Patients With Multiple Vessel Disease (ERACI-II), Medicine, Angioplasty or Surgery Study for Multi-Vessel Coronary Artery Disease (MASS-II), and the Stent or Surgery trial (SoS) showed similar 5-year survival outcomes but higher revascularization rates among patients with bare-metal stents [1,2]. Similarly, a meta-analysis of 23 RCTs by Bravata et al. has reported that survival outcomes up to 10 years were similar between PCI and CABG, although CABG was superior to PCI in that it relieved angina and led to fewer repeated revascularization [3]. Recently, pooled analysis of 10 RCTs by Hlatky et al. reported that long-term mortality is similar after PCI and CABG, although CABG might be a better option for patients with diabetes and those aged 65 years or older in terms of lower mortality [4]. However, all these trials used selected study population which tended to exclude high-risk patients such as with left main disease, the elderly, or left ventricular dysfunction. Thus, their results may not be generalized to current clinical practice [5,6].

On the other hand, several registry data that included more complex patients than RCTs have shown superiority of CABG in comparison to PCI [11–14]. Hannan et al. reported that CABG is associated with better 3-year adjusted survival outcomes than PCI in patients with two or more diseased coronary arteries using the data from the New York Registry, which included approximately 60 000 patients [11]. Similarly, Malenka et al. reported that adjusted survival is better after CABG than that after PCI in patients with triple-vessel disease [12]. Hannan et al. also compared outcomes between PCI using drug-eluting stent and CABG and showed that CABG constitutes to be associated with lower mortality than does treatment with drug-eluting stents, and is associated with lower mortality or myocardial infarction and repeat revascularization [13]. Meta-analysis of observational cohorts by Benedetto et al. also demonstrated that overall major adverse cardiac and cerebrovascular event rate continues to be higher after PCI by drug-eluting stents due to an excess of redo revascularization compared with CABG [14]. These results indicate that survival outcomes are similar between PCI and CABG in low- or moderate-risk patients; however, CABG is associated with better survival outcomes than PCI in high-risk patients [5,6].

PCI versus CABG in multivessel with left main disease

There are few registry data that investigated patients including left main disease. Brener et al. studied 6033 patients with high

risks in which half of the patients had significant LV dysfunction or diabetes [15]. In addition, the study population included approximately 20% patients with left main disease. They showed that PCI was associated with an increased risk of death (propensity-adjusted HR = 2.3, $p < 0.0001$). Left main disease was one of significant independent predictors for mortality ($p < 0.01$). Biryukova et al. reported that CABG is associated with improved major adverse cardiovascular and cerebrovascular events in patients with three-vessel and/or left main stem disease compared with PCI at 6 and 12 months [16]. Recently, a larger RCT of drug-eluting stents versus CABG for left main disease (the Synergy between PCI and Taxus and Cardiac Surgery (SYNTAX) trial) demonstrated that CABG was associated with better outcomes at 1 year proportionally with the increase in SYNTAX score [17]. In patients undergoing CABG, the binary 12-month rates of major adverse cardiac or cerebrovascular events were similar among patients with low (0–22, 14.7%) and those with high scores (>33, 10.9%). By contrast, in patients with PCI, the rate of those events was significantly increased among patients with high SYNTAX scores (23.4%) as compared with those with low scores (13.6%) ($p = 0.002$ for high vs low scores). This result also indicates that CABG is associated with better outcomes than PCI in high-risk patients with more complex coronary lesions, including left main disease. Registry arm of SYNTAX trial also reported that CABG still remains the dominant revascularization strategy in patients with multivessel or left main disease [18].

In our previous report, we could not demonstrate the superiority of CABG in comparison to PCI regarding adjusted survival outcomes ($p = 0.06$) in patients with multivessel disease without left main disease in the CREDO-Kyoto Registry [9]. In the present study, however, we have shown that CABG, particularly OPCAB, is associated with better adjusted survival and event-free outcomes than PCI. Furthermore, OPCAB was associated with better survival outcomes in high-risk subgroups such as those with LV dysfunction and the elderly. The present analysis additionally included patients with left main disease into analysis data set, and the differences in outcomes between the two studies appear to be attributable to inclusion of patients with left main disease. It should be noted that PCI for left main disease was adopted more selectively in the era of bare-mental stent (BMS) as compared with contemporary clinical practice and, therefore, patients with left main disease are more prone to be subjected to selection bias.

Impact of OPCAB on coronary revascularization

Several RCTs and meta-analyses have been conducted over the last decade comparing outcomes of OPCAB and ONCAB. Equivalent short- and long-tem angiographic graft patency has also been demonstrated [19,20]. However, the benefit of OPCAB regarding mortality and morbidity (stroke and myocardial infarction) has been controversial [7,8,20–22]. This may be because these studies have been underpowered to determine significant differences in these endpoints [23]. Recently, a large RCT by Shroyer et al. (The ROOBY trial) reported that patients undergoing OPCAB had worse 1-year composite outcomes (death, myocardial infarction, or repeated revascularization) and poorer graft patency than those undergoing ONCAB [22]. However, the study excluded high-risk patients with small target vessels or diffuse coronary disease. More importantly, most of the operations were performed by relatively inexperienced surgeons. Thus, a study involving surgeons with more experience and high-risk patients will more accurately reflect real-world CABG outcomes.

On the other hand, several large registry data have provided compelling evidence in favor of OPCAB. The New York State Registry reported that OPCAB had significantly lower risk-adjusted 30-day mortality, as well as postoperative stroke and respiratory failure [24]. Survival outcome was similar between ONCAB and OPCAB, although patients undergoing OPCAB needed more repeated revascularization. An intension-to-treat analysis of 42 477 patients from the Society of Thoracic Surgeons National Adult Cardiac database showed a reduction in risk-adjusted mortality, stroke, and preoperative myocardial infarction in patients undergoing OPCAB [25]. In the present study of the CREDO-Kyoto Registry, there were no differences in survival and event-free (myocardial infarction and repeated revascularization) between ONCAB and OPCAB. However, the incidences of stroke and composite cardiovascular event were lower after OPCAB [9].

Study limitations

There are several important limitations of this study. First, this study deals with patients with PCI using bare-metal stents. Further study comparing CABG with PCI using drug-eluting stents will be favorable. Second, important medications, statins in particular, to prevent cardiovascular events are obviously underused. Although inclusion or exclusion of medications did not influence the survival outcomes in the present study, more optimal use of medications might have changed the long-term outcome of both PCI and CABG.

CONCLUSIONS

CABG, particularly OPCAB, is associated with better survival and event-free outcomes than PCI in patients with multivessel and/or left main disease in bare-metal stent era. The incidence of stroke after OPCAB was lower than that after ONCAB and is similar to PCI. OPCAB may be a favorable coronary revascularization strategy, especially in high-risk populations. Further study comparing CABG with drug-eluting stents with longer follow-up is favorable.

Funding

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Conflict of interest: none declared.

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Clinical Characteristics and Outcomes of Japanese Women Undergoing Coronary Revascularization Therapy

Shunsuke Funakoshi, MD; Yutaka Furukawa, MD; Natsuhiko Ehara, MD; Takeshi Morimoto, MD; Shuichiro Kaji, MD; Atsushi Yamamuro, MD; Makoto Kinoshita, MD; Takeshi Kitai, MD; Kitae Kim, MD; Tomoko Tani, MD; Atsushi Kobori, MD; Michihiro Nasu, MD; Yukikatsu Okada, MD; Toru Kita, MD; Takeshi Kimura, MD on behalf of the CREDO-Kyoto Investigators

Background: Limited data are available for gender-based differences in patients undergoing coronary revascularization. This study aimed to identify gender-based differences in risk factor profiles and outcomes among Japanese patients undergoing coronary revascularization.

Methods and Results: The subjects consisted of 2,845 women and 6,843 men who underwent first percutaneous coronary intervention or coronary artery bypass grafting in 2000–2002. The outcome measures were all-cause death, major adverse cardiovascular events (MACE) as the composite of cardiovascular death, myocardial infarction and stroke, and any coronary revascularization. The females were older than the males and more frequently had histories of heart failure, diabetes, hypertension, chronic kidney disease, anemia, and dyslipidemia. Unadjusted survival analysis revealed a significantly lower incidence of any revascularization in women (at 3 years: 28.2% vs. 31.2%, P=0.0037), although no significant gender-based differences were shown in the incidence of all-cause death (at 3 years: 8.8% vs. 8.5%, P=0.37) or MACE (at 3 years: 12.0% vs. 11.5%, P=0.61). Multivariate analysis revealed that female gender was associated with significantly lower risks of any revascularization (relative risk=0.93, 95% confidence interval [CI]=0.88–0.99, P=0.014) and all-cause death (relative risk=0.86, 95%CI=0.77–0.96, P=0.005).

Conclusions: In Japanese patients undergoing first coronary revascularization, the coronary risk factor burden appeared greater in women than in men. Despite the greater modifiable risk factor accumulation, female gender was associated with a lower incidence of repeated revascularization relative to male gender.

Key Words: Coronary artery disease; Outcomes; Revascularization; Risk factors; Women

Many studies have attempted to clarify gender-based differences in the outcomes of patients with coronary artery disease (CAD). Some studies have reported that female patients undergoing coronary revascularization have higher rates of mortality and major complications than men,[1–6] while others have shown that the outcomes of female patients have improved and the gender-based differences of patients undergoing percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) have recently decreased.[7–11] Moreover, there are also a few studies suggesting that the female gender is an independent predictor of better long-term survival after coronary revascularization.[12–14] Gender-based differences in the outcomes of patients with CAD might be attributed, in part, to differences in the clinical backgrounds between female and male patients such as age, risk factor profiles, and comorbid diseases. Other factors that could cause gender-based differences in the outcomes might include unawareness of the importance of secondary prevention for CAD among women.

Most studies regarding gender-based differences in CAD patients have been performed in Caucasian patients. Despite

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Investigators in the Coronary REvascularization Demonstrating Outcome study in Kyoto (CREDO-Kyoto) registry are listed in the Appendix.

Mailing address: Yutaka Furukawa, MD, Department of Cardiovascular Medicine, Kobe City Medical Center General Hospital, 4-6 Minatojima-nakamachi, Chuo-ku, Kobe 650-0046, Japan. E-mail: furukawa@kcho.jp

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