Paclitaxel-Induced Endothelial Dysfunction in Living Rats Is Prevented by Nicorandil via Reduction of Oxidative Stress

Ken-ichi Serizawa¹, Kenji Yogo¹, Ken Aizawa¹, Yoshihito Tashiro¹, Yoko Takahari², Kaori Sekine³, Toshihiko Suzuki⁴, Nobuhiko Ishizuka¹,* and Hideyuki Ishida⁴

¹Product Research Department, Chugai Pharmaceutical Co., Ltd., 1-135 Komakado, Gotemba, Shizuoka 412-8513, Japan
²Teaching and Research Support Center, ³Department of Pediatrics, ⁴Department of Physiology, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan

Received March 18, 2012; Accepted June 14, 2012

Abstract. Paclitaxel-eluting stents dramatically reduce rates of in-stent restenosis; however, paclitaxel is known to lead to endothelial dysfunction. Protective effects of nicorandil on paclitaxel-induced endothelial dysfunction by examining flow-mediated dilation (FMD) were investigated in anesthetized rats. After 7-day osmotic infusion of paclitaxel (5 mg/kg per day), FMD was measured by high-resolution ultrasound in the femoral artery of living rats. Paclitaxel significantly reduced FMD (21.6% ± 3.2% to 7.1% ± 1.7%); this reduction was prevented by co-treatment with nicorandil (15 mg/kg per day), while paclitaxel did not affect nitroglycerin-induced vasodilation. Diazoxide and tempol, but not isosorbide dinitrate, had an effect similar to nicorandil in preventing paclitaxel-induced decrease in FMD. Nicorandil significantly prevented paclitaxel-induced reduction in acetylcholine-induced vasodilation. On the underlying mechanisms, paclitaxel increased reactive oxygen species (ROS) production (dihydrorhodamine 123, DCF fluorescence intensity) and NADPH oxidase (p47phox, gp91phox mRNA) in arteries and human coronary artery endothelial cells (HCAECs), while paclitaxel reduced nitric oxide (NO) release (DAF-2 fluorescence intensity), but not endothelial NO synthase (eNOS) phosphorylation in HCAECs. Nicorandil prevented the increased ROS production in arteries and HCAECs, which was 5-hydroxydecanoate (5-HD)-sensitive but 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ)-resistant, without significant effect on the reduced NO release. In conclusion, nicorandil prevents paclitaxel-induced endothelial dysfunction, which may be brought by improved NO bioavailability due to the reduction of oxidative stress via K_ATP channel activation.

Keywords: endothelial dysfunction, flow-mediated dilation, nicorandil, paclitaxel, reactive oxygen species
cal preconditioning effect (10). It is also reported, both in the clinical setting and in animal studies, that the reduction in cardiac events is due in part to the endothelial protective effects of nicorandil: nicorandil improved flow-mediated dilation (FMD, an indicator of endothelial function) in patients with ischemic heart disease (11) or with cardiovascular risk factors (12) and reduced myocardial no-reflow in swine by protecting endothelial function (13). However, there is no report of nicorandil having a protective effect on paclitaxel-induced endothelial dysfunction.

In the present study, we investigated the protective effect of nicorandil on paclitaxel-induced endothelial dysfunction and also the mechanisms underlying this protective effect in association with NO bioavailability in vascular endothelial cells. In the clinical setting, endothelial function is evaluated by measuring FMD, and FMD has also been used to evaluate endothelial function in dogs and pigs (14, 15). Recently, we reported the method for measurement of endothelial function by FMD in rats (16). Therefore, in this study we measured FMD in the femoral artery of living rats by using high-resolution ultrasound under conditions that maintained blood flow, many humoral factors, and nerve activity.

Materials and Methods

Chemicals

Nicorandil [N-(2-hydroxyethyl)nicotinamide nitrate ester] was synthesized in the Chugai Organic Chemistry Laboratory. Paclitaxel and other reagents used were of analytical grade from Wako Pure Chemicals Co. (Osaka). 4-Hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (tempol), 5-hydroxydecanoate (5-HD), and 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), isosorbide dinitrate (ISDN), and diazoxide were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Nitroglycerin (NTG; Nippon Kayaku, Tokyo) and acetylcholine (Daichi-Sankyo, Tokyo) were purchased. Paclitaxel was dissolved in 50% dimethylformamide, 25% Cremophor EL, and 25% distilled water. Diazoxide and ISDN were suspended in 3% gum arabic solution. NTG and acetylcholine were dissolved in and diluted with 0.9% saline solution, and the other drugs were freshly dissolved in distilled water just before the experiment. The concentrations of paclitaxel used in this study were chosen on the basis of previous studies (7, 17).

Animals

All animal procedures were conducted in accordance with Chugai Pharmaceutical’s ethical guidelines for animal care, and all experimental protocols were approved by the Animal Care Committee of the institution and conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. All animals were euthanized by exsanguination under anesthesia after experiments.

Measurements of endothelial function in live rats

Male Sprague Dawley rats (250 – 300 g) were used. All rats were fed ordinary laboratory chow and allowed free access to water under a constant light and dark cycle of 12 h. Rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg) and adequate anesthetic depth was monitored by pinching the toe. An osmotic pump (model 2ML1; Durect Corp., Cupertino, CA, USA) was implanted subcutaneously into a small pouch between the shoulder blades, with topical application of bupivacaine (5 mg/mL) to the skin wound, for continuous infusion of paclitaxel (0.5, 1.5, 5 mg/kg per day). For the vehicle group, an osmotic pump containing vehicle for paclitaxel (described above) was subcutaneously implanted. Nicorandil (15 mg/kg per day) and tempol (20 mg/kg per day) were dissolved in drinking water and administered for 1 week from just after implantation of the osmotic pump. Diazoxide (15 mg/kg) and ISDN (15 mg/kg) were administrated by gastric gavage once a day for 1 week from just after osmotic pump implantation. FMD was measured in rats 1 week after implantation of the osmotic pump.

FMD was measured in the rats as previously described (16). Briefly, rats were anesthetized with thiobutabarbital (120 mg/kg, i.p.) and adequate anesthetic depth was monitored by pinching the toe. A catheter was inserted into the jugular vein for drug administration, while the body core temperature was kept stable. The femoral arterial diameter and Doppler flow were measured by using a high-resolution ultrasound system with a 30-MHz transducer (Vevo770; VisualSonics, Toronto, Canada). After the femoral artery was identified by its distinctive flow pattern, the position of the probe was optimized to clearly show the vessel wall/lumen interface and was fixed throughout the experiment. Vascular diameter and Doppler flow were obtained from longitudinal sections of the femoral artery before and after 5 min of hind limb ischemia. A snare occluder (5–0 nylon surgical suture passed around the artery and through a 4-cm length of PE-200 tubing) was positioned upstream of the visualized area at the common iliac artery through a transabdominal access. After the common iliac artery was occluded, flow arrest was confirmed by abrogation of the Doppler signal. The changes in flow velocity and diameter of the femoral artery occurring with reperfusion after 5 min of ischemia were monitored until 3 min after reperfusion. After the FMD measurements, NTG (5 μg/kg, i.v.) was injected via the jugular vein catheter to
evaluate endothelium-independent vasodilation. Acetylcholine (3 ng) was injected into the aorta just proximal to the iliac bifurcation to evaluate endothelium-dependent vasodilation.

**Real-time PCR analysis in rat femoral arteries**

After measurement of FMD, femoral arteries were harvested and stored in RNA later solution (Ambion, Austin, TX, USA) until RNA isolation was performed. Tissues were homogenized using a Micro Smash homogenizer (Tomy Digital Biology, Tokyo), and total RNA was isolated using an RNeasy Fibrous Tissue kit (Qiagen, Dusseldorf, Germany). TaqMan real-time PCR was performed using TaqMan Gene Expression Assays for NADPH oxidase components p47phox and gp91phox in an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Carlsbad, CA, USA). Gene expression was normalized to the endogenous control, β-actin.

**Measurement of reactive oxygen species (ROS) in mouse testicular arteries**

In vivo measurement of ROS production monitored by using the fluorescent probe, dihydrodorhamidine 123 hydrochloride (Wako Pure Chemical) in mice was conducted as previously described (18). Briefly, male ICR mice (10–11 week-old) were anesthetized with pentobarbital (50 mg/kg, i.p.). Endothelial cells were observed by FITC-labeled isolectin B4, a murine-specific endothelial cell marker (Vector Lab, Burlingame, CA, USA). After the testicular artery (approx. 200 μm in diameter) was carefully exposed, dihydrorhodamine 123 (2.5 mg/kg) was given intravenously, followed by intravenous administration of FITC-labeled isolectin B4 (1.25 mg/kg). The fluorescence intensity dependent on the increase in ROS was observed by an ultrafast laser confocal microscope equipped with a piezoelectric motor control unit and was recorded as arbitrary units (au). Under pentobarbital anesthesia, mice were subcutaneously implanted with the osmotic pump (model 1007D, Durect Corp.), with body temperature kept stable and monitored for adequate anesthetic depth by the pinching toe of the animal. Paclitaxel (5 mg/kg per day), was continuously infused from the osmotic pump. Nicorandil (15 mg/kg per day) was administrated in drinking water from just after infusion of paclitaxel.

**Measurements of ROS in HCAECs**

HCAECs were purchased from Lonza (Walkersville, MD, USA) and cultured in EBM-2 supplemented with 5% fetal bovine serum. For the measurements of ROS production, the cells were seeded onto plastic dishes (1 × 10^5 cells / 2 mL per dish) and cultured in monolayers in a 5% CO₂ humidified incubator at 37°C. After overnight incubation, paclitaxel (10 ng/mL) was added to the culture medium for 24 h. Nicorandil (100 μM), 5-HD (500 μM), and ODQ (20 μM) were added over the same period.

The cellular level of ROS was monitored by using the fluorescent probe 2’,7’-dichlorodihydrofluorescein diacetate (H₂DCFDA) (Invitrogen, Carlsbad, CA, USA). Oxidation by ROS converts H₂DCFDA to highly fluorescent 2’,7’-dichlorofluorescein (DCF). Briefly, cultured cells were incubated with 10 μM H₂DCFDA for 45 min, and then DCF fluorescence from at least 20 cells/dish, 3 dishes per experimental condition, was quantified with a confocal microscope (Zeiss Axiovert 200; Carl Zeiss Microscopy, Göttingen, Germany) and quantified with the Image J program (version 1.240, http://rsb.info.nih.gov/ij/). The data were obtained with three independent measurements for each experimental condition.

**Measurements of NO in HCAECs**

Intracellular NO was assessed with diaminofluorescein-2 diacetate (DAF-2DA; Sekisui Medical, Tokyo) fluorescence probe (10 μM) (19). Briefly, HCAECs were incubated with PBS containing 10 μM DAF-2 in the dark for 30 min at 37°C and then washed twice with PBS. Fluorescence intensity was next measured by spectrofluorophotometry (SpectraMax Gemini XS; Molecular Devices, Sunnyvale, CA, USA) with excitation and emission wavelengths of 485 and 545 nm, respectively.

**Statistical analyses**

All data are expressed as the mean ± S.E.M. The n values refer to the number of individual animals in which experiments were performed. The statistical significance of differences was determined by the Tukey Multiple Comparison Test. Probability (P) values of less than 0.05 were considered significant.

**Results**

**Measurements of FMD in rats**

In vehicle-treated rats, reperfusion after 5 min of hind limb ischemia led to an instantaneous increase in femoral arterial flow velocity (reactive hyperemia) compared with baseline (Fig. 1: A, B), followed by rapid decay to baseline values at around 3 min (Fig. 1C). Ischemia also caused an increase in the diameter of the rat femoral artery from baseline (Fig. 1D) to 1 min after reperfusion (Fig. 1E). The increase in flow velocity was associated with the delayed increase in femoral arterial vasodilation that peaked at 1 min (Fig. 1F). This delayed vasodilation in the femoral artery is referred to as FMD.
Effects of nicorandil on reduced FMD in paclitaxel-treated rats

Continuous subcutaneous infusion of paclitaxel (0.5, 1.5, 5 mg/kg per day, 7 days) by the implanted osmotic pump reduced FMD in a dose-dependent manner (Fig. 2A), whereas both the increase in peak post-ischemic reactive hyperemia and the NTG-induced vasodilation were similar among all treatment groups (data not shown). These results imply that paclitaxel impairs endothelial function without changing contractility in vascular smooth muscle [Fig. 2A; FMD of control group: 21.6% ± 3.2%; FMD of paclitaxel group (5 mg/kg per day): 7.1% ± 1.7%]. On the other hand, co-treatment with nicorandil prevented the decrease in FMD induced by paclitaxel (FMD of paclitaxel + nicorandil group: 15.5% ± 2.5%) without changing flow velocity or NTG-induced vasodilation (data not shown). We previously reported that nicorandil did not significantly change FMD in normal rats (16). In the current study we found that treatment with paclitaxel and nicorandil for 1 week did not affect bodyweight, blood pressure, heart rate, or pre-occlusion femoral artery diameter as compared with those parameters in vehicle-treated rats (data not shown).

Effects of nicorandil on acetylcholine-induced vasodilation in paclitaxel-treated rats

In vehicle-treated rats, local administration of acetylcholine into the iliac artery led to femoral arterial vasodilation, which peaked at 10 s and rapidly decayed to baseline value by approximately 1 min. In rats treated with paclitaxel (5 mg/kg per day), acetylcholine-induced vasodilation was significantly reduced in the femoral artery (control: 12.7% ± 2.1%; paclitaxel: 3.9% ± 1.1%; \( P < 0.05 \) vs. control). On the other hand, nicorandil significantly prevented the paclitaxel-mediated decrease in acetylcholine-induced vasodilation (Fig. 2B; paclitaxel + nicorandil: 12.5% ± 3.5%; \( P < 0.05 \) vs. paclitaxel).
Endothelial Protection by Nicorandil

Effects of diazoxide, ISDN, and tempol on reduced FMD in paclitaxel-treated rats

Diazoxide, a mitochondrial K$_{ATP}$-channel opener, significantly prevented the decrease in FMD induced by paclitaxel (0.5, 1.5, 5.0 mg/kg per day; 1 week) and the effects of nicorandil (15 mg/kg per day, 1 week) on paclitaxel-induced impairment of FMD (n = 5 – 9). Columns show mean percentage increase (± S.E.M.) in arterial diameter at 1 min after reperfusion. B) Effects of nicorandil on acetylcholine-induced vasodilation (n = 7 – 8). Acetylcholine was injected into the common iliac artery via an intra-aortic catheter. Columns show mean percentage increase (± S.E.M.) in arterial diameter at 10 s after acetylcholine injection. *P < 0.05 vs. control, *P < 0.05 vs. paclitaxel (5 mg/kg per day).

Effects of diazoxide, ISDN, and tempol on reduced FMD in paclitaxel-treated rats

Diazoxide, a mitochondrial K$_{ATP}$-channel opener, significantly prevented the decrease in FMD induced by paclitaxel, although ISDN, a nitrate, had no effect (Fig. 3). Tempol, a ROS scavenger, also significantly prevented the decrease in FMD induced by paclitaxel (Fig. 3). Neither diazoxide, ISDN, nor tempol affected the flow velocity, NTG-induced vasodilation, bodyweight, blood pressure, heart rate, or pre-ischemic femoral artery diameter (data not shown).

Fig. 2. Effects of nicorandil on paclitaxel-induced impairment of endothelial function. Dose-dependent impairment of FMD by paclitaxel (0.5, 1.5, 5.0 mg/kg per day; 1 week) and the effects of nicorandil (15 mg/kg per day, 1 week) on paclitaxel-induced impairment of FMD (n = 5 – 9). Columns show mean percentage increase (± S.E.M.) in arterial diameter at 1 min after reperfusion. B) Effects of nicorandil on acetylcholine-induced vasodilation (n = 7 – 8). Acetylcholine was injected into the common iliac artery via an intra-aortic catheter. Columns show mean percentage increase (± S.E.M.) in arterial diameter at 10 s after acetylcholine injection. *P < 0.05 vs. control, *P < 0.05 vs. paclitaxel (5 mg/kg per day).

Fig. 3. Effects of diazoxide, ISDN, and tempol on the paclitaxel-dependent impairment of endothelial function. Diazoxide (15 mg/kg per day) or ISDN (15 mg/kg per day) was suspended in 3% gum arabic solution and administrated by gastric gavage once a day for 1 week. Tempol (20 mg/kg per day, p.o.) was administrated in drinking water for 1 week. *P < 0.05 vs. control, *P < 0.05 vs. paclitaxel (5 mg/kg per day); n = 5 – 7.

Fig. 4. Effects of nicorandil on paclitaxel-induced changes in expression of p47$^{phox}$ and gp91$^{phox}$ mRNA in rat femoral arteries. p47$^{phox}$ (A) and gp91$^{phox}$ (B) expression levels in femoral arteries were measured using real-time PCR. Data are shown as subunit / β-actin ratio. *P < 0.05 vs. control, *P < 0.05 vs. paclitaxel (5 mg/kg per day); n = 8.
Effects of nicorandil on gene expression of NADPH oxidase in femoral arteries of paclitaxel-treated rats

In femoral arteries isolated from paclitaxel-treated rats, the mRNA expressions of p47phox and gp91phox, the major components of NADPH oxidase, were significantly increased (Fig. 4, $P < 0.05$ vs. control). On the other hand, nicorandil significantly prevented the increase in p47phox expression (Fig. 4A, $P < 0.05$ vs. paclitaxel) and also tended to prevent the increase in gp91phox expression (Fig. 4B, $P = 0.09$ vs. paclitaxel) in the femoral arteries of paclitaxel-treated rats.

Effects of nicorandil on ROS production in testicular arteries of paclitaxel-treated mice

As shown in the Fig. 5, the fluorescence intensity of dihydrorhodamine 123 hydrochloride in paclitaxel-treated mice was 1.6 times greater than that in vehicle-treated mice. On the other hand, co-treatment with nicorandil significantly inhibited the increase in fluorescence intensity induced by paclitaxel (control: 33.0 ± 0.5; paclitaxel: 52.2 ± 1.1, $P < 0.05$ vs. control; paclitaxel + nicorandil: 44.3 ± 1.0 au, $P < 0.05$ vs. paclitaxel).

Effects of nicorandil on ROS production, NO release, and endothelial NO synthase (eNOS) phosphorylation induced by paclitaxel in HCAECs

Paclitaxel (10 ng/mL) significantly increased DCF fluorescence compared with the control. Nicorandil significantly inhibited the increase in DCF fluorescence induced by paclitaxel (Fig. 6, $P < 0.05$ vs. control). This inhibitory effect was abolished by co-treatment with 5-HD, a mitochondrial KATP-channel inhibitor (Fig. 7A), but not by co-treatment with ODQ, a specific inhibitor of soluble guanylate cyclase (Fig. 7B). Treatment with 5-HD alone showed an increase in DCF fluorescence compared with the control (Fig. 7A), whereas treatment with nicorandil or ODQ alone did not affect DCF fluorescence (Fig. 6, 7B). On the other hand, there was no

![image](image_url)
difference in phosphorylation of eNOS protein among the 3 groups.

Paclitaxel (10 ng/mL) significantly decreased DAF-2 fluorescence intensity compared with the control (Fig. 8, \( P < 0.05 \) vs. control), while nicorandil tended to inhibit the decrease in DAF-2 fluorescence intensity induced by paclitaxel. Nicorandil alone had no effect on the fluorescence intensity. On the other hand, there was no significant difference in the phosphorylation of eNOS protein (phosphorylated eNOS / total eNOS ratio) among the vehicle control, paclitaxel (10 ng/mL), and paclitaxel with nicorandil (100 \( \mu \)M) in cultured HCAECs (data not shown).

Discussion

Although paclitaxel-eluting stents (PES) provide dramatic reductions in in-stent restenosis, paclitaxel is known to lead to endothelial dysfunction. We investigated the protective effect of nicorandil on paclitaxel-induced endothelial dysfunction and its underlying
mechanisms by using FMD in anesthetized rats. The main finding of the present study was that paclitaxel-induced endothelial dysfunction in anesthetized rats via both an increase in oxidative stress and a decrease in NO production. Furthermore, based on the results of functional FMD, ROS production, mRNA expression, NO synthesis, and eNOS phosphorylation, the present study also demonstrated that nicorandil would protect against paclitaxel-induced endothelial dysfunction mainly through the inhibition of ROS production.

With respect to the mechanism of paclitaxel-induced endothelial dysfunction, it has been postulated that paclitaxel could reduce NO availability. A critical determinant of endothelial function is the balance between NO and ROS, and it has been judged that the availability of endothelium-derived NO can be limited by enhanced formation of ROS (20, 21). NO can be trapped with ROS in a very rapid reaction, forming peroxynitrite and resulting in the reduction of the amount of available NO (22). An earlier report has indicated that a significant source of the superoxide produced following treatment with paclitaxel is NADPH oxidase and that an NADPH oxidase inhibitor in oxidative stress-induced ROS (20, 21). NO can be trapped with ROS in a very rapid reaction, forming peroxynitrite and resulting in the reduction of the amount of available NO (22). An earlier report has indicated that a significant source of the superoxide produced following treatment with paclitaxel is NADPH oxidase and that an NADPH oxidase inhibitor or suppression of gp91phox by siRNA significantly attenuated paclitaxel-induced cell death (23). Furthermore, reduction of NO availability by paclitaxel may also enhance thrombotic processes because reduced NO availability not only attenuates vasodilation but also adversely affects anti-thrombotic processes such as reduced leukocyte adhesion, platelet adhesion, aggregation, and expression of plasminogen activator inhibitor (PAI-1) (21), and indeed, paclitaxel increased PAI-1 mRNA expression in HCAECs (24). Our findings are consistent with those earlier reports. In this study, paclitaxel increased ROS production both in vivo (Fig. 5) and in HCAECs (Fig. 6), with the concurrent increases in mRNA expression of NADPH oxidase (p47phox and gp91phox, Fig. 4) in the rat. Moreover, tempol prevented the decrease in FMD by paclitaxel in rat femoral arteries (Fig. 3). On the other hand, paclitaxel decreased NO release in HCAECs (Fig. 8), but did not change eNOS phosphorylation. These data clearly suggest that paclitaxel induces endothelial dysfunction by limiting the availability of endothelium-derived NO via both increased ROS production and decreased NO release. Earlier reports indicated that paclitaxel induced endothelial dysfunction in humans (5), animals (6, 25), and cultured cells (7, 26) through apoptosis by increasing the level of pro-apoptotic mRNA transcripts, although the relationship between reduction of NO availability induced by paclitaxel and endothelial dysfunction was not fully revealed. Tawa et al. reported that hypoxia-induced ROS production in smooth muscle cells impaired NO donor-mediated relaxation in endothelium-denuded monkey coronary arteries (27). This means that ROS has the potential to impair not only the NO bioavailability but also the vascular smooth muscle function. However, in this study, NTG-induced relaxation was unimpaired by paclitaxel, implying that paclitaxel-induced ROS production did not impair smooth muscle function in this study. The reasons for this discrepancy may lie in differences in degree of ROS production or in the methods of measurement of vascular relaxation.

Earlier studies suggested that nicorandil had a protective effect on the endothelium through its antioxidative effect. On the basis of experimental results showing that nicorandil reinforced the anti-aggregatory activity of endothelial cells through the reduction of ROS production under hypoxia–reoxygenation conditions, Tajima et al. suggested that the protective effect of nicorandil on the endothelium was mediated by inhibition of NADPH oxidase activity via mitochondrial KATP channel opening (28). Eguchi et al. suggested that nicorandil prevented thrombus formation via activation of mitochondrial KATP channels and inhibition of ROS-induced ROS release in the mitochondria of endothelial cells (18). The present study suggests that nicorandil prevents paclitaxel-induced endothelial dysfunction by reduction of oxidative stress. The action of nicorandil in this study seems to be dependent on its mitochondrial KATP channel opening properties because diazoxide, another mitochondrial KATP-channel opener, also prevented the paclitaxel-induced reduction of FMD (Fig. 3) and because the reduction of ROS by nicorandil was inhibited by 5-HD, a mitochondrial KATP-channel blocker, in HCAECs (Fig. 7A). Interestingly, treatment with 5-HD alone showed an increase in oxidative stress (Fig. 7A), suggesting that the mitochondrial KATP channel is important in the regulation of oxidative stress induced in the endothelium by paclitaxel. Ozcan et al. also demonstrated that nicorandil prevented oxidative stress via opening of the mitochondrial KATP channel (29). Although it cannot be ruled out completely, the action of nicorandil in this study does not seem to be dependent on its nitrate-like activity. In fact, ISDN did not prevent the paclitaxel-induced decrease in FMD (Fig. 3), and ODQ did not inhibit the decrease in ROS by nicorandil in HCAECs (Fig. 7B). Thus, nicorandil might attenuate ROS-induced ROS release elicited by paclitaxel through mitochondrial stabilization.

We chose in vivo measurements of FMD for evaluation of endothelial function instead of in vitro evaluation for the following reasons: Firstly, in most reports endothelial function was evaluated experimentally as acetylcholine-induced vasodilation in isolated arteries; clinically, however, endothelial function is determined at the brachial artery under the influence of nervous innervation and humoral factors within the blood stream. Secondly, endothelial function can be evaluated either by NO avail-
ability or by sensing of shear stress (30). NO availability is determined by NO production depending on the expression level and the enzyme activity of eNOS, both of which are modulated by various humoral factors such as increased ROS (21), increased asymmetric dimethylarginine (ADMA) (21, 31), depletion of tetrahydrobiopterin (32, 33), and increased advanced glycation end products (34). Moreover, blood flow–induced shear stress stimulates NO production from the concerted action of multiple mechanotransducer molecules including the glycocalyx, integrins, and caveolae (30), which can be reduced under certain genetic or pathological conditions.

Measurement of FMD in rats is considered a useful experimental tool for estimating endothelial dysfunction in serious vascular diseases. We previously reported the impairment of FMD in streptozotocin-induced diabetic rats (16) according to the FMD method described in the report by Heiss et al. (35). Endothelial function could be successfully assessed by in vivo FMD measurement in our current experiment because 5-min ischemia/reperfusion or intra-arterial acetylcholine induced femoral vasodilation without affecting the reactive hyperemia or NTG-induced endothelium-independent vasodilation in anesthetized rats. The present study suggests that measurement of FMD in rats provides a useful and reproducible method not only for investigating the side effects of pharmacological treatments, such as endothelial dysfunction induced by paclitaxel used in DES or in cancer therapy (36), but also for evaluating the therapeutic effects on endothelial dysfunction in several models of vascular disease such as hypertension, heart failure, diabetes, and chronic kidney disease.

In the IONA trial, nicorandil significantly improved the prognosis by reducing the incident rates of major coronary events (8). One of the underlying mechanisms for prognosis improvement was explained by pharmacological preconditioning (37). Recent clinical studies have suggested that nicorandil’s protective effects against endothelial dysfunction would be another possible mechanism because endothelial dysfunction is a risk factor for cardiovascular disease. It has been reported that in patients with cardiovascular risk factors, long-term administration of nicorandil improves FMD (11, 12, 38) and that this improvement is due to the reduction of oxidative stress (12, 38). In the current study, nicorandil prevented the paclitaxel-induced decrease of FMD due to the reduction of oxidative stress. Therefore, these results suggest that nicorandil might have favorable prognostic effects against endothelial dysfunction in PES-implanted patients as well as in patients with cardiovascular risk factors. To assess the clinical implications of our findings, further clinical studies are needed.

There is a limitation in the present study. Systemic plasma concentration of paclitaxel would be lower in the clinical setting than that in these experiments, and it remains unclear whether systemically administered paclitaxel has other points of action. However, in arteries isolated from PES-implanted swine, the PES enhanced local oxidative stress, which may contribute to endothelial-dependent vasomotor dysfunction (6); therefore, it is clear that the PES itself causes endothelial dysfunction in the artery downstream of the implantation point.

In conclusion, we demonstrate in rat femoral artery that nicorandil protects against paclitaxel-induced endothelial dysfunction as evaluated by FMD, which may be brought about by improved NO bioavailability due to the reduction of oxidative stress via $K_{ATP}$ channel activation.

References

25 Pires NM, Eefting D, de Vries MR, Quax PH, Jukema JW. Siroli-