INTRODUCTION:
Titanium (Ti) has been widely used as materials of the joint prosthesis for the orthopaedic reconstruction. However, the dissemination of soluble metallic corrosion products and particulate metallic wear debris after the long-term implantation may play an important role in the prosthesis-related late complications. The blood concentration of Ti from human patients or rats with Ti-alloy prostheses has been reported to be elevated after implantation (1, 2). The precise action and mechanism of elevated Ti on the blood vessels and endothelial cells still remain unclear. In the present study, therefore, we intended to explore the in vivo effects of Ti alloy implants on rat blood vessels and in vitro effects of soluble form of Ti on human endothelial cells.

MATERIALS AND METHODS:
The insertion of titanium alloy implants. Wistar rats weighing 200 to 250 g were used. The insertion of titanium alloy implants, the same material as a clinical Ti-alloy prosthesis, was done under pentobarbital anesthesia. The disc-shaped implant had a diameter of 5 mm and was 2 mm thick. All the implants were rinsed in 70 % ethanol in water and were then autoclaved. The implant was placed in the abdominal wall between the peritoneum and the rectus muscle. After 4 weeks, the animals were sacrificed under anesthesia. Western blotting, a 30-50 g sample of each cell lysate was subjected to electrophoresis on 10% SDS-polyacrylamide gels. After blocking, blots were incubated with anti-phospho-eNOS, anti-eNOS, anti-phospho-PKC (pan), anti-PKC-α, and anti-α-tubulin for 1 h. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies for 30 min. Enhanced chemiluminescence reagents were employed to depict the protein bands on membranes. Rat thoracic aortic rings. Rats thoracic aortic rings, 4-5 mm in width, were suspended between two hooks connected to a transducer (Grass FT 03H) for the measurement of isometric force. The aortic rings with intact endothelium were used. These aortic rings were suspended in 10 ml organ baths containing oxygenated (95% O2+5% CO2), warmed (37°C) Krebs solution. Cell Culture. Human umbilical vein endothelial cells (HUVECs) were cultured in medium 199, supplemented with 20 mmol/L HEPES, 100 μg/ml endothelial cell growth substance, and 20 % fetal calf serum. The cultures were maintained at 37°C with gas of 5 % CO2 - 95 % air mixture. In some experiments, titanium dioxide (TiO2) in sulfuric acid was prepared as soluble form of Ti and was adjusted to pH 7.0.

RESULTS:
The maximal contractile responses elicited by phenylephrine in the aortas of rats 4 weeks after the Ti alloy implantation were significantly decreased as compared with sham-control (Fig. 1A). To investigate the alteration of NO synthesis in the aortas of Ti alloy-implanted rats, Western blot analysis was used to detect the change of eNOS protein expression. Ti alloy implant enhanced the expression of eNOS protein in the aortas of rats (Fig.1B). The PKC-α protein expression in membrane fraction of aortas in Ti alloy-implanted rats was also increased (Fig. 1B). Furthermore, we determined the effects of soluble form of Ti on the rat aortas and human endothelial cells. Treatment of rat aortas with Ti (30 μM) for a period of 18 h suppressed the maximal contractile response elicited by phenylephrine (data not shown). Treatment of HUVECs with Ti (10-60 μM) for 24 h could dose-dependently increase the eNOS protein expression (Fig. 2A). HUVECs treated with Ti (30 μM) for 1 h effectively enhanced the phosphorylation of eNOS and PKC (pan), which could be inhibited by PKC inhibitors RO320432 and cherythrine (1 μM). In A, Data are presented as mean±SEM from five independent experiments. *P < 0.05 vs control. In B, Results shown are representative of three independent experiments.

DISCUSSION:
In the present work, we used the isolated rat thoracic aortas with intact endothelium model to investigate the in vivo effects of Ti alloy implants on vasoconstriction and eNOS protein expression, and used the human endothelial cells culture model to test the in vitro effects of soluble form of Ti on eNOS and PKC expressions. The results showed that Ti is capable of enhancing the expression of eNOS in rat aortas and human endothelial cells. The PKC phosphorylation and PKC-α protein expressions in rat aortas and human endothelial cells were also enhanced by Ti. PKC inhibitors could inhibit the increase of PKC phosphorylation and eNOS protein expression by Ti. PKC-α has been shown to possess ability to activate eNOS in endothelial cells and increase blood flow (3). These findings, therefore, indicate that Ti-alloy implants can induce a PKC-activated eNOS/NO-mediated vasodilation. Further study is needed to investigate the clinical significance of this biological effect induced by Ti.

REFERENCES: