calibrator). As a calibrator, we used the average C_t value for untreated chondrocytes. All experiments included negative controls, which consisted of no cDNA for each primer pair.

SA β-gal activity assay. SA β-gal activity was detected by cytochemical staining of chondrocytes using a senescence cells histochemical staining kit (Sigma) according to the manufacturer's instructions. Cells were seeded at 1×10^6/well in a 35-mm plate and allowed to grow to 70% confluence. Chondrocytes were stimulated for 24 hours with the indicated concentrations of ox-LDL or with 100 μg/ml of native LDL. Some plates were preincubated for 30 minutes with 40 μg/ml of TS-20 (an anti–LOX-1 blocking antibody) or with 40 μg/ml of nonspecific mouse IgG and then stimulated for 24 hours with 100 μg/ml of ox-LDL. After stimulation, cells were washed with ice-cold phosphate buffered saline (PBS) and incubated with fixation buffer (1.5 ml/well) for 7 minutes at room temperature. After the fixation process, cells were washed 3 times with PBS and incubated for 12 hours at 37°C with staining mixture (1 mg/well). Blue-stained cells were considered SA β-gal–positive cells.

Assay for the incorporation of 5′-bromo-2′-deoxyuridine (BrdU). The proliferative ability of chondrocytes was evaluated by measuring the incorporation of BrdU into newly synthesized DNA. The incorporation of BrdU in cultured BACs at 70% and 100% confluence and in precultured chondrocytes assessed immediately after isolation were examined using BrdU Labeling and Detection Kit I (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Cells were incubated for 60 minutes at 37°C with 5 ml/well of BrdU-linked medium. The BrdU-linked medium was removed and the cells were washed and fixed for 1 hour at −20°C of 70% ethanol (1 ml/well). After fixation, cells were incubated for 30 minutes at 37°C with 3 ml/well of anti-BrdU reaction mixture and then incubated for 30 minutes with fluorescein isothiocyanate (FITC)–linked anti-mouse immunoglobulin reaction mixture (3 ml/well).

Cells were washed and examined with a confocal laser microscope (LCM5 Pascal Laser Scanning Microscope; Carl Zeiss Instruments, Oberkochen, Germany). The incorporation of BrdU into BACs cultured with various agents was quantified using a cell proliferation enzyme-linked immunosorbent assay (ELISA; Roche Diagnostics) according to the manufacturer's instructions. Chondrocytes were seeded in a Falcon 96-multiwell plate at a density of 2×10^4/well in 100 μl of culture medium. After incubation, 10 μl of BrdU solution was added to each well, and the plate was incubated for 2 hours at 37°C. After incubation, the culture medium was removed, the cells were fixed, and DNA was denatured by the addition of 200 μl/well of FixDenat for 1 hour. The reagent with peroxidase-labeled anti-BrdU antibody was added to the cells, and the plate was incubated for 90 minutes. At the end of the reaction time, the substrate was added for 5 minutes, and the absorbance at 370 nm and 492 nm (control) was measured with a microplate reader (Model 680; Bio-Rad, Hercules, CA).

Telomerase activity assay. Telomerase activity per 2×10^4 cells was measured by a stretch PCR method using TeloChaser (Toyobo, Osaka, Japan), according to the manufacturer's instructions. The cells were washed with PBS, and the cell pellet was resuspended in the lysis solution that came with the kit and then centrifuged at 15,000 revolutions per minute for 20 minutes. The supernatant was subjected to telomerase reactions at 37°C for 30 minutes. The products were denatured at 95°C for 2 minutes 30 seconds, amplified by PCR (33 cycles at 95°C for 30 seconds, 68°C for 30 seconds, and 72°C for 45 seconds) using Platinum *Taq* polymerase (Invitrogen, Auckland, New Zealand), subjected to electrophoresis on a 10% polyacrylamide gel in Tris−borate−EDTA buffer for 60 minutes at 100V, stained with 0.5 μg/ml of ethidium bromide for 15 minutes, and visualized with a blot detection system (Printgraph AE-6932; Atto, Tokyo, Japan). The signal intensities were quantified using image analysis software (ImageJ version 1.37; NIH Image, National Institutes of Health, Bethesda, MD; online at: http://rsbweb.nih.gov/ij/).

Western blot analysis. Chondrocytes were washed with ice-cold PBS, lysed in lysis buffer (sample buffer solution, Wako Pure Chemical Industries), and boiled for 5 minutes. The solution obtained was subjected to immunoblotting. Cell lysates were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and the proteins were transferred onto a polyvinylidene fluoride membrane. After blocking, the membrane was incubated overnight at 4°C with the primary antibody, incubated with the secondary antibody for 1 hour, and then visualized with an ECL Plus Western blot detection system (Amersham Biosciences, Little Chalfont, UK). The signal intensities were measured using NIH ImageJ software, version 1.37, as above.

Statistical analysis. Results are presented as the mean ± SD. Analyses of variance, Scheffe's tests, and Student's unpaired *t*-tests were used for statistical assessments. *P* values less than 0.05 were considered statistically significant.

RESULTS

Expression of matrix mRNA in cultured BACs. First, we investigated the expression of matrix genes in cultured BACs using real-time PCR analysis. Real-time PCR showed that the primary cultured BACs constitutively expressed mRNA for aggrecan and type II collagen, indicating that the cells did not dedifferentiate prior to stimulation by the agents. The expression of aggrecan and type II collagen mRNA in 70% confluent BACs was 7.6 ± 1.7–fold higher (mean ± SD) and 8.5 ± 1.8–fold higher, respectively, than in precultured chondrocytes that were assessed immediately after isolation. The expression of aggrecan and type II collagen mRNA was significantly higher in 70% confluent BACs than in 100% confluent BACs (data not shown).

Effects of ox-LDL on SA β-gal activity. To assess the effects of ox-LDL on chondrocyte senescence, SA β-gal cytochemical staining of cultured BACs was performed after 24 hours' incubation with ox-LDL (0, 50, or 100 μg/ml) or native LDL (100 μg/ml). Treatment with ox-LDL increased the ratio of SA β-gal–positive (blue-stained) cells and the intensity of the staining in a dose-dependent manner (Figures 1A–C), whereas native LDL did not increase the staining intensity (Figure 1D).

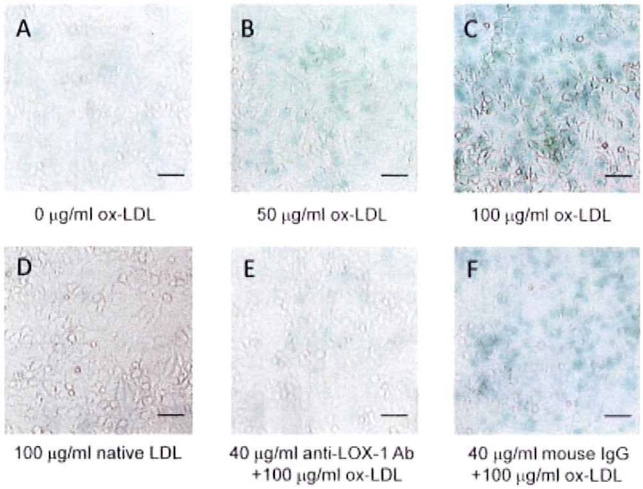

Figure 1. Effects of oxidized low-density lipoprotein (ox-LDL) on senescence-associated (SA) β-galactosidase (β-gal) activity in cultured bovine articular chondrocytes (BACs). SA β-gal activity in cultured BACs was investigated by cytochemical staining. Chondrocytes were incubated for 24 hours with 0 μg/ml of ox-LDL (**A**), 50 μg/ml of ox-LDL (**B**), 100 μg/ml of ox-LDL (**C**), or 100 μg/ml of native LDL (**D**). Some plates were pretreated for 30 minutes with 40 μg/ml of TS-20, an anti–lectin-like ox-LDL receptor 1 (anti–LOX-1) antibody (Ab) (**E**), or with 40 μg/ml of nonspecific mouse IgG (**F**) and then incubated with 100 μg/ml of ox-LDL. SA β-gal–positive cells are stained blue. Bars = 20 μm.

To examine the receptor specificity of the ox-LDL, we pretreated the BACs for 30 minutes with TS-20 (40 μg/ml), an anti–LOX-1 blocking antibody, before ox-LDL stimulation. The ox-LDL–induced increase in SA β-gal staining was significantly attenuated by pretreatment with TS-20 (Figure 1E) but not by pretreatment with 40 μg/ml of nonspecific mouse IgG (Figure 1F).

Effects of ox-LDL on the ability of cultured BACs to proliferate. The effects of ox-LDL on the proliferative ability of cultured BACs were investigated by measuring BrdU incorporation, as observed by fluorescence staining and as quantified by ELISA. Fluorescence images revealed that the preincubated chondrocytes tested immediately after isolation were BrdU negative and that more BrdU-positive BACs were observed in the 70% confluent BACs than in the 100% confluent BACs (data not shown). ELISA demonstrated that the mean BrdU incorporation was 6.3-fold higher in the 70% confluent BACs than in the preincubated chondrocytes. BrdU incorporation was significantly higher in the 70% confluent BACs than in the 100% confluent BACs (data not shown).

To examine whether ox-LDL affects the ability of cultured BACs at 70% confluence to proliferate, chondrocytes were incubated for 24 hours with ox-LDL or native LDL at various concentrations (0, 10, 50, or 100 μg/ml), and BrdU incorporation was quantified by ELISA. Chondrocytes preincubated with 40 μg/ml of TS-20 (anti–LOX-1 blocking antibody) or with 40 μg/ml of nonspecific mouse IgG for 30 minutes were also stimulated with the indicated doses of ox-LDL. Addition of ox-LDL suppressed BrdU incorporation into cultured BACs in a dose-dependent manner (Figure 2A), but native LDL did not (Figure 2B). Pretreatment with TS-20 recovered the ox-LDL–induced suppression of

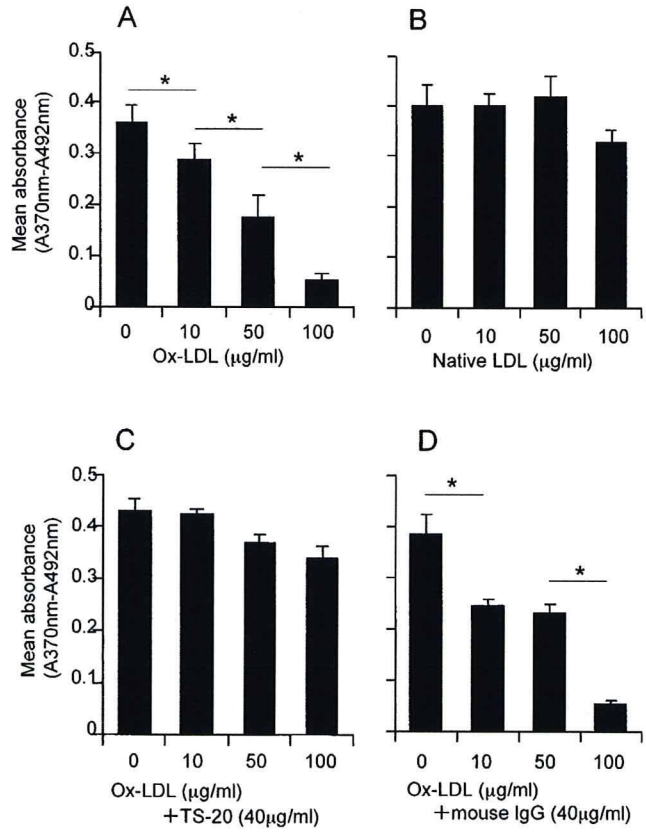

Figure 2. Effects of ox-LDL on the proliferative ability of cultured 70% confluent BACs. The proliferative ability of cultured BACs in 70% confluent cultures was quantified by bromodeoxyuridine incorporation using an enzyme-linked immunosorbent assay. Chondrocytes were incubated for 24 hours with the indicated concentrations of ox-LDL (**A**) or native LDL (**B**), or chondrocytes were pretreated for 30 minutes with 40 μg/ml of TS-20 (**C**) or with 40 μg/ml of nonspecific mouse IgG (**D**) and were then stimulated with the indicated concentrations of ox-LDL. Values are the mean and SD of 4 experiments per condition. * = $P < 0.01$. See Figure 1 for definitions.

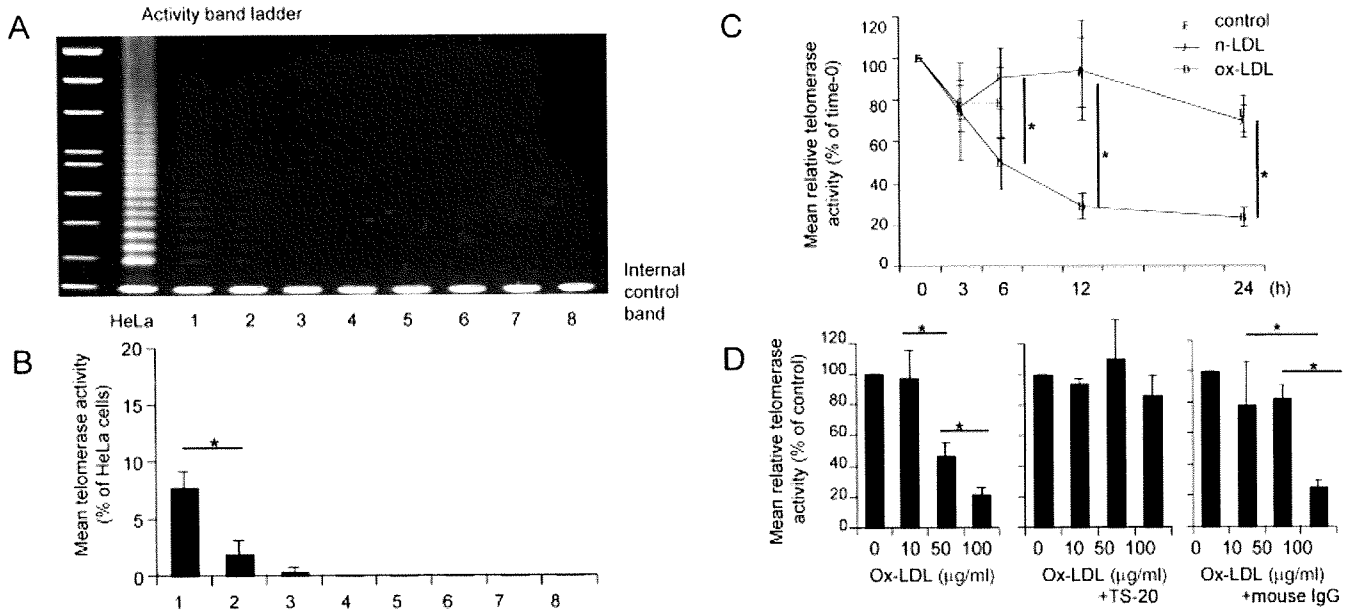

Figure 3. Telomerase activity in cultured BACs and time and dose effects of ox-LDL on telomerase activity. **A,** Telomerase activity was measured by a stretch polymerase chain reaction (PCR) method in positive control cells (HeLa), cultured BACs at 70% confluence (lane 1), cultured BACs at 100% confluence (lane 2), preincubated BACs (lane 3), heat-treated HeLa cells (lane 4), heat-treated BACs at 70% confluence (lane 5), heat-treated BACs at 100% confluence (lane 6), heat-treated, preincubated BACs (lane 7), and negative control (lane 8). Heat-treated samples were incubated at 85°C for 10 minutes. **B,** The relative telomerase activity was quantified with image analysis software, and the results for lanes 1–8 in **A** are shown. Compared with HeLa cells, the mean telomerase activity was 7.8% in 70% confluent BACs and 1.9% in 100% confluent BACs. **C,** BACs were incubated with culture medium (control), 50 μg/ml of native-LDL (n-LDL), or 50 μg/ml of ox-LDL, and telomerase activity was measured at the indicated times using a stretch PCR method and image analysis software. Thick vertical bars indicate the comparison groups with significant differences. **D,** BACs were incubated for 12 hours with the indicated concentrations of ox-LDL. BACs preincubated for 30 minutes with 40 μg/ml of anti–LOX-1 antibody (TS-20) or 40 μg/ml of nonspecific mouse IgG were also stimulated with various doses of ox-LDL. Values in **B–D** are the mean and SD of 4 experiments per condition. * = $P < 0.01$. See Figure 1 for other definitions.

BrdU incorporation (Figure 2C), but pretreatment with nonspecific mouse IgG did not (Figure 2D).

Telomerase activity of cultured BACs and effects of ox-LDL. Telomerase activity was lower in cultured BACs at 70% and 100% confluence than in HeLa cells but was detectable using a stretch PCR method (Figure 3A); relative to the activity in HeLa cells, the mean ± SD values were 7.8 ± 1.4% for 70% confluent cells and 1.9 ± 1.2% for 100% confluent cells (n = 4) (Figure 3B). Activity was barely detectable in the precultured chondrocytes (0.3 ± 0.6% of the values in the HeLa cells; n = 4) (Figure 3B).

We next investigated the effects of ox-LDL on the telomerase activity of cultured BACs at 70% confluence. BACs were incubated with 50 μg/ml of native LDL or ox-LDL for various times (0, 6, 12, or 24 hours). Incubation with ox-LDL, but not native LDL, suppressed telomerase activity in a time-dependent manner (Figure 3C). We further investigated the dose effects of ox-LDL on the telomerase activity of BACs. Chondrocytes preincubated for 30 minutes with 40 μg/ml of anti–LOX-1 blocking antibody (TS-20) or nonspecific mouse IgG were also stimulated with the indicated doses of ox-LDL. Telomerase activity was suppressed by ox-LDL in a dose-dependent manner. Pretreatment with TS-20 significantly reversed this suppression, but nonspecific mouse IgG had no effect (Figure 3D).

Effects of ox-LDL on the PI3K/Akt pathway. To clarify the mechanism of action of ox-LDL in the suppression of telomerase activity, we investigated the relationship between the PI3K/Akt pathway and the telomerase activity of BACs. BACs were stimulated for 12 hours with various concentrations of the PI3K inhibitor LY294002 (0, 0.05, 0.5, or 1 n*M*). Some wells were stimulated simultaneously with 100 ng/ml of IGF-1 (an activator of PI3K) and the indicated doses of ox-LDL for 12 hours. Incubation with LY294002 but without ox-LDL suppressed the telomerase activity of BACs in a

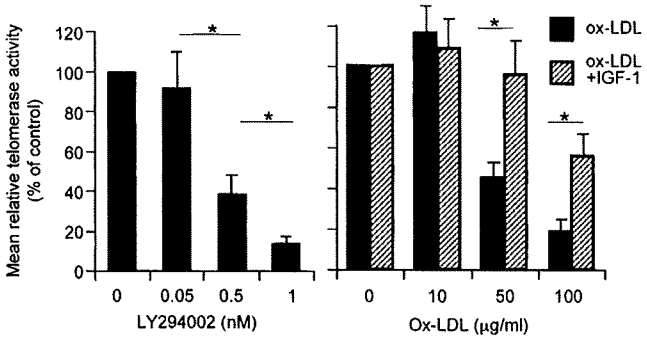

Figure 4. Effects of LY294002 (an inhibitor of phosphatidylinositol 3-kinase [PI3K]) and insulin-like growth factor 1 (IGF-1; an activator of PI3K) on telomerase activity. Bovine articular chondrocytes were stimulated for 12 hours with the PI3K inhibitor LY294002 at the indicated concentrations. Some wells were simultaneously stimulated for 12 hours with the PI3K activator IGF-1 (100 ng/ml) and with oxidized low-density lipoprotein (ox-LDL) at the indicated concentrations. Values are the mean and SD of 4 experiments per condition. * = $P < 0.01$.

dose-dependent manner. Addition of IGF-1 resulted in recovery of the suppressed telomerase activity that had been induced by ox-LDL (Figure 4).

Next, we used Western blot analysis to investigate whether ox-LDL affects the PI3K/Akt pathway in cultured BACs. Oxidized LDL at a concentration of 50 μg/ml rapidly decreased the amount of pAkt but had no effect on the amount of Akt. Native LDL did not affect the amount of pAkt. Pretreatment with 40 μg/ml of anti–LOX-1 blocking antibody (TS-20) significantly reversed the ox-LDL–induced decrease in pAkt levels, but nonspecific mouse IgG did not (Figure 5A). Treatment with LY294002 (0.5 n*M*) rapidly decreased the amount of pAkt in BACs, and 100 ng/ml of IGF-1 reversed the ox-LDL–induced decrease in pAkt levels (Figure 5B).

DISCUSSION

The telomere hypothesis is generally accepted as the explanation for the mechanism that underlies cell senescence (26,27). Structures of telomeres, the terminal, guanine-rich sequences of chromosomes (TTAGGG repeats in humans and other vertebrates), work to stabilize the chromosome during replication by protecting the chromosome end against exonucleases. Telomere length decreases with replication, and when this has decreased to a critical length, the cell is signaled to stop dividing and to enter cellular senescence (replicative senescence) (26,27). Telomerase is an RNA-dependent DNA polymerase that synthesizes telomeric DNA sequences.

Telomerase consists of 2 essential components. One is the functional RNA component (called hTR, or hTERC, in humans), which serves as a template for telomeric DNA synthesis. The other is a catalytic protein with reverse transcriptase activity (hTERT) and the primary determinant for the enzyme activity (28,29).

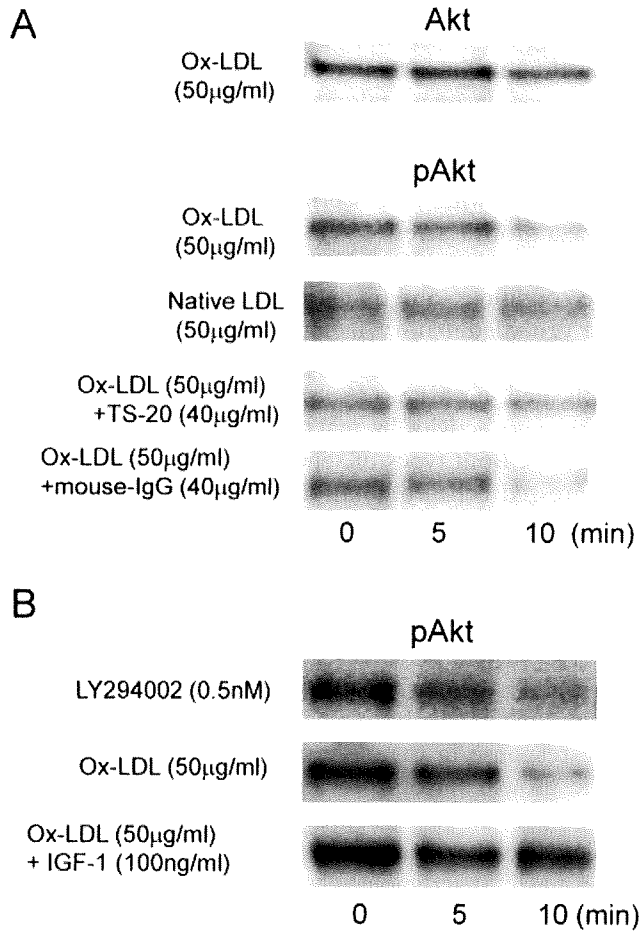

Figure 5. Effects of oxidized low-density lipoprotein (ox-LDL), LY294002 (an inhibitor of phosphatidylinositol 3-kinase [PI3K]), and insulin-like growth factor 1 (IGF-1; an activator of PI3K) on the amounts of pAkt in bovine articular chondrocytes (BACs). **A,** The amounts of Akt and pAkt were determined by Western blotting at the indicated times after stimulation with 50 μg/ml of ox-LDL or native LDL. BACs pretreated with 40 μg/ml of TS-20 (an anti–lectin-like ox-LDL receptor 1 antibody) or nonspecific mouse-IgG were also stimulated with 50 μg/ml of ox-LDL. **B,** The amount of pAkt was determined by Western blotting at the indicated times after stimulation with 0.5 n*M* LY294002 or 50 μg/ml of ox-LDL. Some wells were also stimulated simultaneously with 100 ng/ml of IGF-1 and 50 μg/ml of ox-LDL. All experiments were performed 4 times, and the results were similar.

Although hTERT is generally repressed in normal somatic cells, telomerase activation in human vascular smooth muscle cells protects telomere shortening with replication (30). Because vascular cell senescence occurs in human atherosclerotic lesions and is associated with telomere shortening, telomerase activity seems to be important in guarding against cell senescence (21). Telomere shortening has been demonstrated in OA chondrocytes (23,31,32), and the lifespan of senescent chondrocytes retrieved from OA cartilage can be increased by the exogenous expression of telomerase (33), indicating an important relationship between chondrocyte senescence and telomerase activity.

In addition to replicative senescence, stress-induced premature cell senescence (SIPS) also occurs; in SIPS, cells without discernible attrition of telomeres show a growth arrest (34,35). Some stressors identified include DNA damage, oxidative stress, suboptimal culture conditions, and inhibition of PI3K. Proatherogenic and proinflammatory factors, such as ox-LDL, tumor necrosis factor α, and hydrogen peroxide, have been implicated in SIPS (34,35), and these can suppress telomerase activity by inactivating the PI3K/Akt pathway (36). Both types of senescence are associated with suppressed cell proliferation and impaired physiologic cell function. It is likely that both types of cell senescence, the telomere shortening–initiated and the stress-induced, may contribute jointly to the pathogenic process of chronic diseases in vivo (34).

Clusters or clones of proliferating chondrocytes surrounded by newly synthesized matrix molecules constitute one of the histologic hallmarks of the chondrocyte response in the early phase of OA (37–39). Anabolic growth factors previously trapped in the matrix may be released in a process of matrix degradation, which activates chondrocytes to proliferate and synthesize matrix macromolecules (40). These factors in the synovial fluid may have better access to chondrocytes because of fissuring or loosening of the collagen network or because of damage to the collagen matrix itself (37). These phenomena are thought to represent repair responses of damaged cartilage. The progressive degeneration of cartilage in the later phase of OA may be attributed to limited repair responses caused by cell senescence associated with reduced cell function and proliferative ability (22,40). Chondrocytes in OA cartilage show distinctive features of the senescent cell, including higher activity of SA β-gal, reduced matrix production and proliferative abilities, and telomere shortening, indicating a strong relationship between chondrocyte senescence and cartilage degeneration (22,23,32).

In the present study, we first investigated the properties of the cultured BACs. Real-time PCR results revealed greater expression of aggrecan and type II collagen mRNA in proliferating 70% confluent chondrocytes than in 100% confluent chondrocytes, which had stopped proliferating because of contact inhibition, and in preincubated chondrocytes assessed immediately after isolation. The proliferative ability, as evaluated by BrdU uptake, was also greater in the 70% confluent chondrocytes than in the 100% confluent chondrocytes and the chondrocytes assessed immediately after isolation. These data suggest that the 70% confluent chondrocytes stimulated by fetal bovine serum have a phenotype similar to that of activated and proliferating chondrocytes in the early phase of OA.

We next investigated whether ox-LDL induces cell senescence of cultured BACs. SA β-gal activity has been recognized to be an important biologic marker of cell senescence (41), and it is higher in cloned chondrocytes in OA cartilage (23,32). Oxidized LDL increased the number of SA β-gal–positive BACs in a dose-dependent manner, but native LDL had no effect. Oxidized LDL reduced the cell proliferative ability, as evaluated by BrdU incorporation, in a dose-dependent manner. Pretreatment with anti–LOX-1 blocking antibody (TS-20) cancelled these effects of ox-LDL on BACs. Similar results have been reported in studies of endothelial progenitor cells (42). The induction of cell senescence caused by ox-LDL occurred within 24 hours and did not need subculturing, indicating that ox-LDL can induce SIPS in chondrocytes.

The "telomere hypothesis" is generally accepted as the explanation for replicative cell senescence. Telomerase is activated in proliferating cells of tissues under repair, which prolongs the cellular replicative capacity and postpones cell senescence (43–45). The regulation of telomerase activity is thought to play an important role in tissue repair and regeneration. We first investigated whether the telomerase activity of BACs changes with the culture conditions that induced distinct proliferating activity in chondrocytes. Compared with the telomerase activity of HeLa cells, the precultured chondrocytes assessed immediately after isolation showed little telomerase activity, 100% confluent chondrocytes showed 2% activity, and 70% confluent chondrocytes showed 8% activity. These results indicate that cultured chondrocytes with a higher proliferating activity have a higher telomerase activity, although the activity in all chondrocytes was lower than in HeLa cells. This

suggests that the telomerase activity in BACs is up-regulated during cell expansion. This result is consistent with the findings of previous reports on the telomerase activity of cultured chondrocytes (46,47) and proliferating somatic cells (48,49). Taken together, these data indicate that the telomerase activity in proliferating and cloning chondrocytes in the early phase of OA is up-regulated and plays an important role in tissue repair by postponing cell senescence and maintaining cell function.

We next investigated the effects of ox-LDL on the telomerase activity of the 70% confluent BACs. Telomerase activity was suppressed in a time-dependent manner to ~20% of the preincubation level after 24 hours of culture and in a dose-dependent manner to 40% of the control level 12 hours after the addition of 50 μg/ml of ox-LDL. This suppressive effect on the telomerase activity was reversed by pretreatment with the LOX-1 blocking antibody TS-20, indicating that ox-LDL suppresses telomerase activity through its receptor LOX-1. Oxidized LDL probably impairs the tissue repair of degenerative cartilage in the early phase of OA, since suppression of telomerase activity in proliferating cells results in telomere shortening and instability, leading to cell senescence. The telomere length of chondrocytes is relatively short in OA cartilage, and decreasing telomere length correlates strongly with increasing expression of SA β-gal and decreasing mitotic activity (23,32).

We also investigated the intracellular signaling pathway by which ox-LDL alters telomerase activity. Telomerase activity is regulated by phosphorylation of the reverse transcriptase (hTERT), and protein kinase C or protein kinase B (Akt) plays a critical role in the phosphorylation of hTERT (50). In general, the PI3K/Akt pathway plays important roles in the progress of the cell cycle, cell proliferation, regulation of nuclear transcription factors, cell survival (51,52), and chondrocyte differentiation and apoptosis (53). Activation of this pathway increases the production of aggrecan (54), and inactivation of this pathway suppresses cell viability in articular chondrocytes (11). Interestingly, Breitschopf et al (36) reported that ox-LDL suppresses telomerase activity by inactivating the PI3K/Akt pathway in endothelial cells.

We found that ox-LDL and LY294002 (a specific inhibitor of PI3K) suppressed the telomerase activity in a dose-dependent manner and that IGF-1 (an activator of PI3K) recovered the ox-LDL–induced suppression of telomerase activity. In addition, ox-LDL reduced the amount of pAkt without changing the amount of Akt, and pretreatment with the anti–LOX-1 blocking antibody recovered the ox-LDL–induced reduction in pAkt levels. Taken together, these results suggest that ox-LDL–induced suppression of telomerase activity can be attributed to inactivation of the PI3K/Akt pathway through binding to LOX-1 (Figure 6).

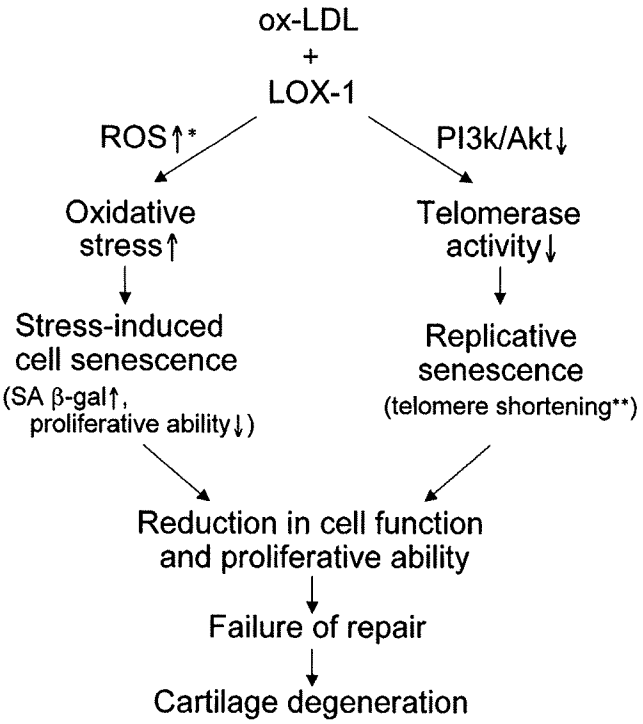

Figure 6. Flowchart summarizing the results of the present study and the assumptive relevance of the oxidized low-density lipoprotein (ox-LDL)/lectin-like ox-LDL receptor 1 (LOX-1) system to the pathogenesis of osteoarthritis. Production of reactive oxygen species (ROS) caused by ox-LDL binding to LOX-1 (*) was reported in our previous study (see ref. 12). Telomere shortening due to ox-LDL (**) is under investigation at our institution. PI3K = phosphatidylinositol 3-kinase; SA = senescence-associated; β-gal = β-galactosidase.

Our study has some important limitations because it was based on an in vitro model using cultured BACs. In this model, the activity of telomerase was only 8% of the HeLa activity, and then, we examined the effects of ox-LDL on this low telomerase activity. Furthermore, the telomerase activity in elderly people with OA may be even lower than the activity described in this study. The results of this study should be interpreted with caution. It is also unclear whether the ox-LDL–induced reduction in this low telomerase activity has any pathologic relevance in OA. However, considering the fact that chondrocytes proliferate in the early stage of OA and the fact that the telomere length of

chondrocytes is actually shortened in OA chondrocytes, telomerase activity seems to play some role in the maintenance of cell function and in the facilitation of tissue repair. We believe that long-lasting and chronic suppression of the telomerase activity in chondrocytes, even if it is initially at a low level, could result in an accumulated failure of tissue repair and a slow progression of cartilage degeneration.

An interesting question is whether proliferation of chondrocytes in the ox-LDL–added medium causes telomere shortening by suppressing telomerase activity, since previous studies have shown a correlation between telomere shortening and cell senescence (23,32). Another interesting question is whether ox-LDL activates the pathways that are linked mechanically to replicative senescence and SIPS, including the ataxia–telangiectasia mutated (ATM)/p53/p21/retinoblastoma (Rb) pathway and the p38/MAPK/p16/Rb pathway (34,35), respectively. We are especially interested in whether adding ox-LDL stabilizes p53, because a recent study showed that p53 destabilizes and permeabilizes lysosomes to shift β-gal from the lysosomes to the cytosol, which is recognized as cytosolic staining of SA β-gal (55).

In conclusion, our data show that ox-LDL binding to LOX-1 induces SIPS in chondrocytes and results in the suppression of telomerase activity through inactivation of the PI3K/Akt pathway. Oxidized LDL may play an important role in the pathogenesis of OA by inducing chondrocyte senescence.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Zushi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Zushi, Akagi, Sawamura.
Acquisition of data. Zushi, Akagi, Kishimoto, Teramura.
Analysis and interpretation of data. Zushi, Akagi, Hamanishi.

REFERENCES

1. Witztum JL, Steinberg D. Role of oxidized low-density lipoprotein in atherogenesis. J Clin Invest 1991;88:1785–92.
2. Sawamura T, Kume N, Aoyama T, Moriwaki H, Hoshikawa H, Aiba Y, et al. An endothelial receptor for oxidized low-density lipoprotein. Nature 1997;386:73–7.
3. Mehta JL, Chen J, Hermonat PL, Romeo F, Novelli G. Lectin-like, oxidized low-density lipoprotein receptor-1 (LOX-1): a critical player in the development of atherosclerosis and related disorders. Cardiovasc Res 2006; 69:36–45.
4. Stockwell RA. Lipid in the matrix of ageing articular cartilage. Nature 1965;207:427–8.
5. Lippiello L. The association of lipid abnormalities with tissue pathology in human osteoarthritic articular cartilage. Metabolism 1991;40:571–6.
6. Tiku ML, Shah R, Allison GT. Evidence linking chondrocyte lipid peroxidation to cartilage matrix protein degradation: possible role in cartilage aging and the pathogenesis of osteoarthritis. J Biol Chem 2000;30:20069–76.
7. Shah R, Raska K Jr, Tiku ML. The presence of molecular markers of in vivo lipid peroxidation in osteoarthritic cartilage: a pathogenic role in osteoarthritis. Arthritis Rheum 2005;52:2799–807.
8. Hart DJ, Doyle DV, Spector TD. Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. J Rheumatol 1995;22:1118–23.
9. McAlindon TE, Jacques P, Zhang Y, Hannan MT, Aliabadi P, Weissman B, et al. Do antioxidant micronutrients protect against the development and progression of knee osteoarthritis? Arthritis Rheum 1996;39:648–56.
10. Nakagawa T, Akagi M, Hoshikawa H, Chen M, Yasuda T, Mukai S, et al. Lectin-like oxidized low-density lipoprotein receptor 1 mediates leukocyte infiltration and articular cartilage destruction in rat zymosan-induced arthritis. Arthritis Rheum 2002;46: 2486–94.
11. Nakagawa T, Yasuda T, Hoshikawa H, Shimizu M, Kakinuma T, Chen M, et al. LOX-1 expressed in cultured rat chondrocytes mediates oxidized LDL-induced cell death: possible role of dephosphorylation of Akt. Biochem Biophys Res Commun 2002;229: 91–7.
12. Nishimura S, Akagi M, Yoshida K, Hayakawa S, Sawamura T, Munakata H, et al. Oxidized low-density lipoprotein (ox-LDL) binding to lectin-like ox-LDL receptor-1 (LOX-1) in cultured bovine articular chondrocytes increases production of intracellular reactive oxygen species (ROS) resulting in the activation of NF-κB. Osteoarthritis Cartilage 2004;12:568–76.
13. Akagi M, Ueda A, Teramura T, Kanata S, Sawamura T, Hamanishi C. Oxidized LDL binding to LOX-1 enhances MCP-1 expression in cultured human articular chondrocytes. Osteoarthritis Cartilage 2009;17:271–5.
14. Kakinuma T, Yasuda T, Nakagawa T, Hiramitsu T, Akiyoshi M, Akagi M, et al. Lectin-like oxidized low-density lipoprotein receptor 1 mediates matrix metalloproteinase 3 synthesis enhanced by oxidized low-density lipoprotein in rheumatoid arthritis cartilage. Arthritis Rheum 2004;50:3495–503.
15. Akagi M, Kanata S, Mori S, Itabe H, Sawamura T, Hamanishi C. Possible involvement of the oxidized low-density lipoprotein/lectin-like oxidized low-density lipoprotein receptor-1 system in pathogenesis and progression of human osteoarthritis. Osteoarthritis Cartilage 2007;15:281–90.
16. Simopoulou T, Malizos KN, Tsezou A. Lectin-like oxidized low density lipoprotein receptor 1 (LOX-1) expression in human articular chondrocytes. Clin Exp Rheumatol 2007;25:605–12.
17. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises. Part I. Aging arteries: a "set up" for vascular disease. Circulation 2003;107:139–46.
18. Felson DT. Epidemiology of osteoarthritis. In: Brandt KD, Doherty M, Lohmander LS, editors. Osteoarthritis. Oxford: Oxford University Press; 1998. p. 13–22.
19. Buckwalter JA, Martin J, Mankin HJ. Synovial joint degeneration and the syndrome of osteoarthritis. Instr Course Lect 2000;49: 481–9.
20. Erusalimsky JD, Kurz DJ. Cellular senescence in vivo: its relevance in ageing and cardiovascular disease. Exp Gerontol 2005; 40:634–42.
21. Minamino T, Komuro I. Vascular cell senescence: contribution to atherosclerosis. Circ Res 2007;100:15–26.
22. Martin JA, Buckwalter JA. The role of chondrocyte senescence in the pathogenesis of osteoarthritis and in limiting cartilage repair. J Bone Joint Surg Am 2003;85 Suppl 2:106–10.
23. Price JS, Waters JG, Darrah C, Pennington C, Edwards DR,

Donell ST, et al. The role of chondrocyte senescence in osteoarthritis. Aging Cell 2002;1:57–65.
24. Martin JA, Klingelhutz AJ, Moussavi-Harami F, Buckwalter JA. Effects of oxidative damage and telomerase activity on human articular cartilage chondrocyte senescence. J Gerontol A Biol Sci Med Sci 2004;59:324–37.
25. Yudoh K, Nguyen T, Nakamura H, Hongo-Masuko K, Kato T, Nishioka K. Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function. Arthritis Res Ther 2005;7:R380–91.
26. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990;345:458–60.
27. Allsopp RC, Vaziri H, Patterson C, Goldstein S, Younglai EV, Futcher AB, et al. Telomere length predicts replicative capacity of human fibroblasts. Proc Natl Acad Sci U S A 1992;89:10114–8.
28. Bryan TM, Cech TR. Telomerase and the maintenance of chromosome ends. Curr Opin Cell Biol 1999;11:318–24.
29. Meyerson M. Role of telomerase in normal and cancer cells. J Clin Oncol 2000;18:2626–34.
30. Minamino T, Mitsialis SA, Kourembanas S. Hypoxia extends the life span of vascular smooth muscle cells through telomerase activation. Mol Cell Biol 2001;21:3336–42.
31. Martin JA, Buckwalter JA. Telomere erosion and senescence in human articular cartilage chondrocytes. J Gerontol A Biol Sci Med Sci 2001;56:B172–9.
32. Martin JA, Buckwalter JA. Human chondrocyte senescence and osteoarthritis. Biorheology 2002;39:145–52.
33. Piera-Velazquez S, Jimenez SA, Stokes DG. Increased life span of human osteoarthritic chondrocytes by exogenous expression of telomerase. Arthritis Rheum 2002;46:683–93.
34. Chen J, Goligorsky MS. Premature senescence of endothelial cells: Methusaleh's dilemma. Am J Physiol Heart Circ Physiol 2006;290: H1729–39.
35. Ben-Porath I, Weinberg RA. The signals and pathways activating cellular senescence. Int J Biochem Cell Biol 2005;37:961–76.
36. Breitschopf K, Zeiher AM, Dimmeler S. Pro-atherogenic factors induce telomerase inactivation in endothelial cells through an Akt-dependent mechanism. FEBS Lett 2001;493:21–5.
37. Sandell LJ, Aigner T. Articular cartilage and changes in arthritis: an introduction: cell biology of osteoarthritis [review]. Arthritis Res 2001;3:107–13.
38. Aubrey JH. Pathology of osteoarthritis. In: Moskowitz RW, Altman RD, Hochberg MC, Buckwalter JA, Goldberg VM, editors. Osteoarthritis. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 51–72.
39. Sandell LJ, Heinegard D, Herring TM. Cell biology, biochemistry, and molecular biology of articular cartilage in osteoarthritis. In: Moskowitz RW, Altman RD, Hochberg MC, Buckwalter JA, Goldberg VM, editors. Osteoarthritis. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 73–106.
40. Buckwalter JA, Mankin HJ, Grodzinsky AJ. Articular cartilage and osteoarthritis. Instr Course Lect 2005;54:465–79.
41. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A 1995;92:9363–7.
42. Imanishi T, Hano T, Sawamura T, Nishio I. Oxidized low-density lipoprotein induces endothelial progenitor cell senescence, leading to cellular dysfunction. Clin Exp Pharmacol Physiol 2004;31: 407–13.
43. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. Science 1994;266:2011–5.
44. Osanai M, Tamaki T, Yonekawa M, Kawamura A, Sawada N. Transient increase in telomerase activity of proliferating fibroblasts and endothelial cells in granulation tissue of the human skin. Wound Repair Regen 2002;10:59–66.
45. Wege H, Brummendorf TH. Telomerase activation in liver regeneration and hepatocarcinogenesis: Dr. Jekyll or Mr. Hyde? Curr Stem Cell Res Ther 2007;2:31–8.
46. Parsch D, Fellenberg J, Brummendorf TH, Eschlbeck AM, Richter W. Telomere length and telomerase activity during expansion and differentiation of human mesenchymal stem cells and chondrocytes. J Mol Med 2004;82:49–55.
47. Parsch D, Brummendorf TH, Richter W, Fellenberg J. Replicative aging of human articular chondrocytes during ex vivo expansion. Arthritis Rheum 2002;46:2911–6.
48. Broccoli D, Young JW, Lange T. Telomerase activity in normal and malignant hematopoietic cells. Proc Natl Acad Sci U S A 1995;92:9082–6.
49. Masutomi K, Yu EY, Khurts S, Ben-Porath I, Currier JL, Metz GB, et al. Telomerase maintains telomere structure in normal human cells. Cell 2003;114:241–53.
50. Liu JP. Studies of the molecular mechanisms in the regulation of telomerase activity. FASEB J 1999;13:2091–104.
51. Jeong SJ, Dasgupta A, Jung KJ, Um JH, Burke A, Park HU, et al. PI3K/AKT inhibition induces caspase-dependent apoptosis in HTLV-1-transformed cells. Virology 2008;370:264–2.
52. Datta SR, Brunet A, Greenberg ME. Cellular survival: a play in three Akts. Genes Dev 1999;13:2905–27.
53. Oh CD, Chun JS. Signaling mechanisms leading to the regulation of differentiation and apoptosis of articular chondrocytes by insulin-like growth factor-1. J Biol Chem 2003;278:36563–71.
54. Starkman BG, Cravero JD, Delcarlo M, Loeser RF. IGF-1 stimulation of proteoglycan synthesis by chondrocytes requires activation of the PI 3-kinase pathway but not ERK MAPK. Biochem J 2005;389:723–9.
55. Yuan XM, Li W, Dalen H, Lotem J, Kama R, Sachs L, et al. Lysosomal destabilization in p53-induced apoptosis. Proc Natl Acad Sci U S A 2002;99:6286–91.

Temporal Pattern of Strokes After On-Pump and Off-Pump Coronary Artery Bypass Graft Surgery

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Background. The incidence of strokes has not decreased after coronary artery bypass graft surgery (CABG). The purpose of this study is to identify incidence, risk factors, and temporal pattern of strokes after on-pump and off-pump CABG.

Methods. We analyzed 2,516 consecutive patients who underwent first elective isolated CABG. The primary endpoint was strokes within 30 days. The temporal onset of the deficits was classified by consensus as either an "early stroke," which is present just after emergence from anesthesia, or a "delayed stroke," which is present after first awaking from surgery without a neurologic deficit.

Results. More than half of strokes (29 of 46; 63%) were delayed strokes. Patients undergoing off-pump CABG had significantly lower risk of early stroke (0.1% versus 1.1%, $p = 0.0009$), whereas the incidence of delayed strokes was not different significantly (0.9% versus 1.4%, $p = 0.3484$) between patients undergoing on-pump and off-pump CABG. In multivariate analyses, undergoing off-pump CABG was an independent protective factor for all strokes (relative risk 0.29, 95% confidence interval: 0.14 to 0.56, $p = 0.0005$) and early strokes (relative risk 0.05, 95% confidence interval: 0.003 to 0.24, $p < 0.0001$), but it was not an independent protective factor for delayed strokes (relative risk 0.54, 95% confidence interval: 0.24 to 1.17, $p = 0.1210$).

Conclusions. Undergoing off-pump CABG reduces the incidence of perioperative stroke mainly by minimizing early strokes; however, the risk of delayed strokes is not different between patients undergoing on-pump and off-pump CABG.

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Despite advances in surgical techniques and improvements in perioperative care, the incidence of perioperative strokes after cardiac surgery has not decreased, and observation that reflects the aging of the population and an increase in the number of elderly patients with coexisting conditions who undergo cardiac surgery. Perioperative strokes result in prolonged hospital stay, increased disability rates, discharge to long-term care facilities, and death after surgery [1]. Many studies have previously compared off-pump coronary artery bypass graft surgery (CABG) with on-pump CABG surgery, and many of these studies have revealed that off-pump CABG has superior outcomes, particularly with regard to short-term mortality and complication rates, including strokes [2–15]. However, how off-pump CABG reduces the incidence of strokes is unclear.

Previous studies have demonstrated that perioperative strokes are predominantly ischemic and embolic, and the timing of embolic strokes after surgery shows a bimodal distribution. Approximately half of the perioperative strokes are identified within the first day after surgery [16, 17]; these events result from manipulations of the heart and aorta or from the release of particulate matter from the cardiopulmonary bypass pump [1, 16, 18]. The remaining half occur after uneventful recovery from anesthesia [16, 17]; these strokes are often attributed to postoperative atrial fibrillation, myocardial infarction, and coagulopathy [18]. Therefore, an investigation into the temporal pattern of strokes has important implications for risk stratification and modification of strokes after CABG.

The purpose of this study is to identify the incidence and risk factors of strokes, including strokes detected early and those detected at a later stage after CABG, and to examine the temporal pattern of strokes according to the type of surgical procedure.

Material and Methods

The Coronary Revascularization Demonstrating Outcome Study in Kyoto (CREDO-Kyoto) registry has collected in-

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hospital and long-term data (median follow-up period, 42.8 months) on the potential risk factors and outcomes in 2,516 consecutive patients who underwent their first isolated CABG at 21 institutions between 2000 and 2002 in Japan. Patients who had had an acute myocardial infarction within 1 week before the index procedure were excluded. The details of the CREDO-Kyoto registry design and main outcomes have been published [19]. The study protocol is concordant with the guidelines for epidemiologic studies issued by the Ministry of Health, Labor, and Welfare of Japan. The relevant review boards or ethics committees in all 21 participating centers approved the research protocol.

In each center, preoperative baseline characteristics and intraoperative data were collected from hospital charts or databases by independent clinical research coordinators according to prespecified definitions. Data in this registry include patient demographics (for example, age and sex), potential risk factors, comorbidities (for example, history of stroke, hypertension, and current smoking status), and intraoperative data (such as internal thoracic artery utilization) that have been demonstrated to be related to clinical outcomes.

To identify the incidence, risk factors, and temporal pattern of strokes, including strokes detected early after on-pump or off-pump CABG, we performed post hoc analysis. Patients were categorized into off-pump CABG and on-pump CABG groups according to the operation that they ultimately underwent. Patients undergoing surgery for cardiac valves or aortic disease simultaneously with CABG were excluded from this study. All procedural decisions and adjunctive pharmacotherapy were made at the discretion of the patient and the surgeon performing CABG, and either off-pump or on-pump CABG was performed at the discretion of the surgeons.

The primary endpoint was stroke occurrence within 30 days, and the temporal onset of the deficits was classified by consensus as follows: "early stroke," if new neurologic deficit was discovered when the patient emerged from anesthesia, and "delayed stroke," if the patient had a neurologic deficit after first awakening from surgery without a neurologic deficit.

Follow-up data after discharge were obtained from hospital charts or by contacting patients or referring physicians. An independent clinical events committee adjudicated events.

Definition of Stroke

Stroke was defined as any new permanent global or focal neurologic deficit that could not be attributed to other neurologic (for example, dementia) or medical (namely, metabolic abnormalities, hypoxia, or drugs) processes. Reversible cerebral ischemic events (transient ischemic attacks, which were defined clinically by the temporary nature of the associated neurologic symptoms that last less than 24 hours) were not included in the analysis because the occurrence of these events cannot be identified under general anesthesia, and their detection is hindered postoperatively by the residual effects of anesthetics, analgesics, and sedatives. In the majority of patients, strokes were diagnosed by neurologists and confirmed by computed tomography or magnetic resonance imaging head scans.

Table 1. Preoperative Baseline Characteristics and Intraoperative Variables of Patients Undergoing On-Pump and Off-Pump Coronary Artery Bypass Graft Surgery

	Overall (n = 2,516)		On Pump (n = 1,399)		Off Pump (n = 1,117)		
	n	%	n	%	n	%	*p* Value
History of stroke	562	22.3%	251	17.9%	311	27.8%	<0.0001
Hypertension	1768	70.3%	930	66.5%	838	75.0%	<0.0001
LVEF ≤40%	264	10.5%	175	12.5%	89	8.0%	<0.0001
Age, years	67.3 ± 9.5		68.6 ± 9.4		66.3 ± 9.5		<0.0001
Peripheral arterial disease	493	19.6%	239	17.1%	254	22.7%	0.0005
Hyperlipidemia	1345	53.5%	715	51.1%	630	56.4%	0.0089
Previous myocardial infarction	846	33.6%	493	35.2%	353	31.6%	0.0558
Serum creatinine ≥2.0 mg/dL	431	17.1%	225	16.1%	206	18.4%	0.0984
Current smoking status	621	24.7%	360	25.7%	261	23.4%	0.1238
Aneurysm	146	5.8%	74	5.3%	72	6.4%	0.2300
Female sex	704	28.0%	384	27.4%	320	28.6%	0.5315
Diabetes mellitus	1155	45.9%	647	46.2%	508	45.5%	0.7473
Dialysis	125	5.0%	69	4.9%	56	5.0%	0.9267
Atrial fibrillation	145	5.8%	80	5.7%	65	5.8%	0.9316
Internal thoracic artery use	2358	93.7%	1346	96.2%	1012	90.6%	<0.0001
Number of anastomoses	3.3 ± 1.1		3.3 ± 1.0		3.2 ± 1.2		0.0047
Emergent procedure	154	6.1%	78	5.6%	76	6.8%	0.2095
CPB time, minutes			123.1 ± 40.1				
Aorta clamp time, minutes			72.5 ± 33.7				

CPB = cardiopulmonary bypass; LVEF = left ventricular ejection fraction.

Table 2. Discharge Medication Regimens of Patients Undergoing On-Pump and Off-Pump Coronary Artery Bypass Graft Surgery

	Overall (n = 2,516)		On Pump (n = 1,399)		Off Pump (n = 1,117)		
	n	%	n	%	n	%	*p* Value
Aspirin	2089	83.0%	1097	78.4%	992	88.8%	<0.0001
Warfarin	749	29.8%	568	40.6%	181	16.2%	<0.0001
Statin	503	20.0%	208	14.9%	295	26.4%	<0.0001
Beta-blocker	249	9.9%	127	9.1%	119	10.7%	0.0005
ACEI/ARB	532	21.1%	242	17.3%	290	26.0%	<0.0001
Type I AAD	423	16.8%	275	19.7%	148	13.2%	<0.0001
Type III AAD	3	0.1%	7	0.5%	10	0.9%	0.1025

AAD = antiarrhythmia drug; ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin-receptor antagonist.

Diagnosis of Associated Conditions

Documentation of a prior stroke required verification by each patient's primary care physician, review of medical records, and review of the results of computed tomography and magnetic resonance imaging if available. Diabetes mellitus or hypertension was considered to be present if the patients were previously diagnosed, or if they were being treated with either insulin or oral antidiabetic drugs or antihypertensive drugs. Patients were considered to have a history of myocardial infarction if infarction had been previously diagnosed on electrocardiographs or coronary angiography. The criteria for the diagnosis of perioperative myocardial infarction were the appearance of new Q waves and an increase in creatine kinase to 2.0 times or more the upper limit of normal occurring 24 hours or less after CABG.

Table 3. Univariate Relationships of Clinical Variables With All Strokes

Variable	Relative Risk	95% CI	*p* Value
Off-pump CABG	0.39	0.18–0.74	0.0065
History of stroke	5.13	2.85–9.44	<0.0001
Atrial fibrillation	4.17	1.86–8.45	0.0002
Age[a]	1.03	1.00–1.07	0.0604
Peripheral arterial disease	1.82	0.93–3.36	0.0660
Hyperlipidemia	1.64	0.90–3.10	0.1113
Hypertension	1.75	0.88–3.89	0.1329
Current smoking status	1.49	0.78–2.75	0.2109
Internal thoracic artery utilization	0.53	0.22–1.54	0.2176
Diabetes mellitus	1.41	0.79–2.55	0.2505
Aneurysm	1.56	0.46–3.93	0.4018
Serum creatinine ≥ 2.0 mg/dL	1.33	0.62–2.61	0.4259
Previous myocardial infarction	1.27	0.69–2.29	0.4343
Emergent procedure	1.44	0.44–8.90	0.6134
LVEF ≤ 40%	0.81	0.24–2.04	0.6970
Number of anastomoses[b]	0.96	0.74–1.23	0.7293
Female	0.91	0.45–1.71	0.7728
Dialysis	0.87	0.14–2.86	0.8452

[a] Hazard ratio for 1 increase in age. [b] Hazard ratio for 1 increase in the number of anastomoses.

CABG = coronary artery bypass graft surgery; CI = confidence interval; LVEF = left ventricular ejection fraction.

Peripheral arterial disease was considered to be present when patients were being treated for carotid, aortic, or other peripheral vascular diseases or were scheduled for surgical or endovascular interventions. Left ventricular ejection fraction (LVEF) was measured either by contrast left ventriculography or echocardiography. Atrial fibrillation contained paroxysmal, persistent, and permanent atrial fibrillation.

Statistical Analysis

Statistical analysis of categorical variables was carried out using cross tables with the Pearson χ^2 test. Survival curves were estimated using the Kaplan-Meier method. To determine the baseline risk factors for the incidence of all strokes, early strokes, and delayed strokes, we developed a Cox proportional hazard model for the following potential variables: off-pump CABG, emergency procedure, history of stroke, atrial fibrillation, aneurysm, peripheral arterial disease, hypertension, age, LVEF 40% or less, hyperlipidemia, serum creatinine greater than 2.0 mg/dL, history of myocardial infarction, current smoking status, diabetes mellitus, dialysis, female sex, internal thoracic artery utilization, and number of anastomoses. All statistical tests were two-tailed; a *p* value less than 0.05 was considered statisti-

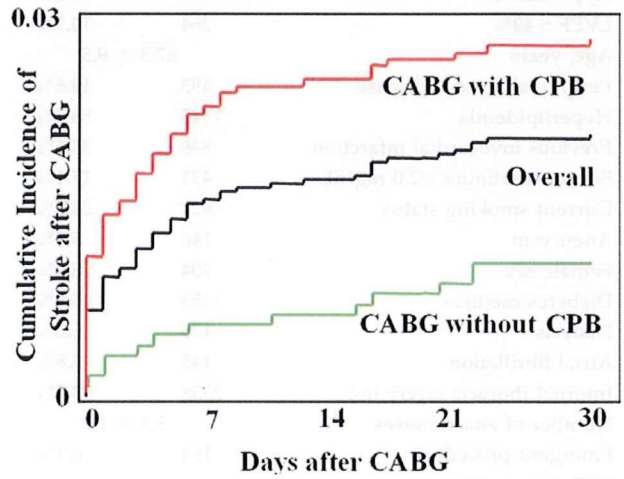

Fig 1. Cumulative incidence of stroke after coronary artery bypass graft surgery (CABG). Postoperative day 0 refers to the day of surgery. (CPB = cardiopulmonary bypass.)

Table 4. Number and Temporal Pattern of Strokes Based on Type of Surgical Procedure

	All Strokes		Early Strokes		Delayed Strokes	
	n	%	n	%	n	%
Overall (n = 2516)	46	1.8%	17	0.7%	29	1.1%
On-pump CABG (n = 1,399)	35	2.5%	16	1.1%	19	1.4%
Off-pump CABG (n = 1,117)	11	1.0%	1	0.1%	10	0.9%
p Value	0.0043		0.0009		0.3484	

CABG = coronary artery bypass graft surgery.

cally significant, and a *p* value less than 0.1 was considered to indicate a statistical tendency. The variables for which *p* values were less than 0.1 in univariate analyses were included in multivariate analyses. All analyses were performed with JMP version 6.0.3 (SAS Institute, Cary, NC).

Results

Preoperative baseline characteristics and intraoperative variables of patients undergoing on-pump and off-pump CABG are shown in Table 1. As indicated, patients undergoing on-pump CABG appeared to have a higher prevalence of reduced left ventricular function (LVEF 40% or less) and internal thoracic artery utilization. Patients undergoing on-pump CABG had also tendency to have history of myocardial infarction more frequently. Conversely, patients undergoing off-pump CABG were older, had a history of stroke more frequently, had lower average number of anastomoses, and showed greater prevalence of hypertension, peripheral arterial disease, and hyperlipidemia. Patients undergoing off-pump CABG also had tendency to have chronic kidney disease (serum creatinine greater than 2.0 mg/dL) more frequently. The discharge medication regimens are shown in Table 2.

All patients continued to attend follow-up examinations at 30 days. Within 30 days after CABG, 46 patients (1.8%) had a stroke. Univariate analyses indicated that undergoing off-pump CABG, history of stroke, and atrial fibrillation were significant predictors of stroke (Table 3).

The number and temporal pattern of strokes based on the type of surgical procedure is shown in Figure 1 and Table 4. The incidence of stroke was sustained for 30 days after CABG. Eighteen strokes were detected early after surgery (37% of strokes, 0.7% of the 2,516 patients); 29 strokes were delayed (63% of strokes, 1.1% of patients). Among patients who underwent off-pump CABG (n = 1,117), 1 stroke occurred early after surgery (9% of strokes, 0.1% of patients); 10 strokes were delayed (91% of strokes, 0.9% of patients). Among patients who underwent on-pump CABG (n = 1,399), 16 strokes were detected early after surgery (46% of strokes, 1.1% of patients); 19 strokes were delayed (54% of strokes, 1.4% of patients; Table 4, Fig 1). Compared with patients undergoing off-pump CABG, patients undergoing on-pump CABG more frequently had early strokes. However, the incidence of delayed strokes did not differ between patients undergoing on-pump and off-pump CABG.

Multivariate analyses (considering the baseline characteristics and results of univariate analyses) indicated that undergoing off-pump CABG was an independent protective factor for stroke, and history of stroke and atrial fibrillation were independent risk factors for stroke (Table 5). Furthermore, undergoing off-pump CABG was an independent protective factor for early stroke, and history of stroke and age was an independent risk factor of early stroke. History of stroke and atrial fibrillation were independent risk factors for delayed stroke (Table 5).

Within 30 days after CABG, 3 and 8 deaths occurred among patients with early and delayed strokes, respectively. This 30-day mortality rate (early strokes, 18%; delayed strokes, 28%) was higher than that observed among patients without perioperative stroke (1.9%, $p < 0.0001$). Perioperative Q-wave myocardial infarction incidence (on-pump 1.5% versus off-pump 1.2%, $p = 0.4704$) did not differ between patients undergoing on-pump and off-pump CABG.

Comment

The temporal pattern of strokes based on the type of surgical procedure was a novel finding of this study. More than half of the strokes (29 of 46; 63%) occurred after initial, uneventful neurologic recovery from cardiac surgery and were defined as delayed strokes. Patients undergoing off-pump CABG had significantly lower risk of early stroke compared with patients undergoing on-pump CABG. In contrast, the incidence of delayed stroke did not significantly differ between patients undergoing on-pump and off-pump CABG. Multivariate analyses in this study also demonstrated that undergoing off-pump CABG was an independent protective factor for all strokes and early

Table 5. Multivariate Relationships of Clinical Variables With All, Early, and Delayed Strokes

Variables	Relative Risk	95% CI	*p* Value
All strokes			
Off-pump CABG	0.29	0.14–0.56	0.0005
History of stroke	5.18	2.80–9.74	<0.0001
Atrial fibrillation	3.28	1.42–6.87	0.0029
Age[a]	1.03	0.99–1.07	0.1393
PAD	1.26	0.63–2.41	0.4991
Early strokes			
Off-pump CABG	0.05	0.003–0.24	<0.0001
History of stroke	7.27	2.67–21.69	0.0001
Age[a]	1.11	1.03–1.19	0.0029
Atrial fibrillation	2.82	0.61–9.46	0.1619
PAD	0.55	0.12–1.79	0.3415
Delayed strokes			
Off-pump CABG	0.54	0.24–1.17	0.1210
History of stroke	4.03	1.86–8.86	0.0005
Atrial fibrillation	3.54	1.26–8.53	0.0189
PAD	1.84	0.81–3.99	0.1395
Age[a]	0.99	0.96–1.04	0.7936

[a] Hazard ratio for 1 increase in age.

CABG = coronary artery bypass graft surgery; CI = confidence interval; PAD = peripheral arterial disease.

strokes, but not for delayed strokes. From this study, we conclude that undergoing off-pump CABG may reduce the incidence of perioperative stroke mainly by minimizing early strokes; however, the risk of delayed strokes does not differ between patients undergoing on-pump and off-pump CABG.

Early strokes are mainly caused by manipulations of the heart and aorta or by the release of particulate matter from the cardiopulmonary bypass pump [1, 16, 18]. Some studies that used transcranial doppler ultrasonography demonstrated the production of aortic emboli on cannulation and application of aortic clamps [20–22] and the production of large quantities of aortic emboli during cardiopulmonary bypass without manipulation of the aorta [23]. Aortic manipulation was also reported to be an independent risk factor for postoperative stroke. Indeed, in this study, only 1 stroke (9% of strokes, 0.1% of patients) was detected early after surgery among patients who underwent off-pump CABG of this study (n = 1,117). Thus, it follows that among patients undergoing off-pump CABG, the incidence of early strokes could be reduced by avoiding cardiopulmonary bypass or by minimizing the manipulation of the aorta. Moreover, for reducing the incidence of stroke, it is important that the surgical technique is selected according to the patient's risk profile.

In this study, the risk of delayed stroke did not significantly differ between patients undergoing on-pump and off-pump CABG. Delayed stroke, which is often attributed to postoperative atrial fibrillation, myocardial infarction, and coagulopathy, remains a problem after both on-pump and off-pump CABG. Multivariate analyses of this study also indicated that atrial fibrillation was independent risk factors for delayed stroke. Atrial fibrillation, which was reported to occur in 30% to 50% of patients after cardiac surgery and to increase the risk of perioperative stroke in some studies [16, 18, 24–28], was found to be a significant predictor of delayed strokes after CABG in this study. No controlled trials have specifically addressed the use of anticoagulation therapy for new-onset postoperative atrial fibrillation; however, the American College of Chest Physicians recommends the consideration of heparin therapy for patients in whom atrial fibrillation develops after surgery, and the continuation of anticoagulation therapy for 30 days after the return of a normal sinus rhythm [29]. It was reported that the incidence of postoperative atrial fibrillation and stroke may be reduced by the prophylactic administration of amiodarone and beta-blockers before cardiac surgery [30]. Because, in this study, we do not have precise information about the in-hospital adjunctive pharmacotherapy that might affect the incidence of perioperative stroke, we excluded the information about the adjunctive pharmacotherapy from the analyses of perioperative strokes. Further studies are required to investigate whether these pharmacologic interventions reduce the incidence of delayed strokes.

Study Limitations

This study was not a randomized observational study. We had no precise information about the mechanism of the strokes, in-hospital adjunctive pharmacotherapy, and the incidence of atrial fibrillation after surgery. Previously, it was reported that delayed stroke also may be related to intimal injury to the ascending aorta due to clamping [31]; however, we also do not have precise intraoperative information (for example, use of intra-aortic balloon pump, intraoperative echocardiography of the ascending aorta, use of single or multiple applications of the aortic cross-clamp) that might affect the incidence of both early and delayed strokes. The study population was not very large, and hence, we could not properly examine the precise predictors of stroke and the protective effects of risk modification to prevent stroke and morbidity.

In conclusion, this is the first study of a large prospective cohort analyzing the temporal pattern of perioperative strokes based on the type of surgical procedure in patients undergoing CABG. We found that more than half of the perioperative strokes after CABG were delayed strokes that occurred after initial uneventful neurologic recovery from surgery. This study also demonstrated that undergoing off-pump CABG might reduce the incidence of perioperative stroke, mainly by minimizing early strokes, when neurologic deficit was detected after the patient's emergence from anesthesia; the risk of delayed strokes did not differ between patients undergoing on-pump and off-pump CABG. Delayed stroke, which is often attributed to postoperative atrial fibrillation, myocardial infarction, and coagulopathy, remains a problem after both on-pump and off-pump CABG.

References

1. McKhann GM, Grega MA, Borowicz LM, Baumgartner WA, Selnes OA. Stroke and encephalopathy after cardiac surgery: an update. Stroke 2006;37:562–71.
2. Benetti FJ, Naselli G, Wood M, Geffner L. Direct myocardial revascularization without extracorporeal circulation. Experience in 700 patients. Chest 1991;100:312–6.
3. Hart JC, Spooner TH, Pym J, et al. A review of 1,582 consecutive Octopus off-pump coronary bypass patients. Ann Thorac Surg 2000;70:1017–20.
4. Calafiore AM, Di Mauro M, Contini M, et al. Myocardial revascularization with and without cardiopulmonary bypass in multivessel disease: impact of the strategy on early outcome. Ann Thorac Surg 2001;72:456–63.
5. Cleveland JC, Shroyer AL, Chen AY, Peterson E, Grover FL. Off-pump coronary artery bypass grafting decreases risk-adjusted mortality and morbidity. Ann Thorac Surg 2001;72:1282–9.
6. Hernandez F, Cohn WE, Baribeau YR, et al. In-hospital outcomes of off-pump versus on-pump coronary artery bypass procedures: a multicenter experience. Ann Thorac Surg 2001;72:1528–34.
7. van Dijk D, Nierich AP, Jansen EW, et al. Early outcome after off-pump versus on-pump coronary bypass surgery: results from a randomized study. Circulation 2001;104:1761–6.
8. Angelini GD, Taylor FC, Reeves BC, Ascione R. Early and midterm outcome after off-pump and on-pump surgery in Beating Heart Against Cardioplegic Arrest Studies (BHACAS 1 and 2): a pooled analysis of two randomised controlled trials. Lancet 2002;359:1194–9.
9. Van Dijk D, Jansen EW, Hijman R, et al. Cognitive outcome after off-pump and on-pump coronary artery bypass graft surgery: a randomized trial. JAMA 2002;287:1405–12.
10. Legare JF, Buth KJ, King S, et al. Coronary bypass surgery performed off pump does not result in lower in-hospital morbidity than coronary artery bypass grafting performed on pump. Circulation 2004;109:887–92.
11. Cheng DC, Bainbridge D, Martin JE, Novick RJ. Does off-pump coronary artery bypass reduce mortality, morbidity, and resource utilization when compared with conventional

coronary artery bypass? A meta-analysis of randomized trials Anesthesiology 2005;102:188–203.
12. Wijeysundera DN, Beattie WS, Djaiani G, et al. Off-pump coronary artery surgery for reducing mortality and morbidity: meta-analysis of randomized and observational studies. J Am Coll Cardiol 2005;46:872–82.
13. Panesar SS, Athanasiou T, Nair S, et al. Early outcomes in the elderly: a meta-analysis of 4921 patients undergoing coronary artery bypass grafting—comparison between off-pump and on-pump techniques. Heart 2006;92:1808–16.
14. Sedrakyan A, Wu AW, Parashar A, Bass EB, Treasure T. Off-pump surgery is associated with reduced occurrence of stroke and other morbidity as compared with traditional coronary artery bypass grafting: a meta-analysis of systematically reviewed trials. Stroke 2006;37:2759–69.
15. Hannan EL, Wu C, Smith CR, et al. Off-pump versus on-pump coronary artery bypass graft surgery: differences in short-term outcomes and in long-term mortality and need for subsequent revascularization. Circulation 2007;116:1145–52.
16. Bucerius J, Gummert JF, Borger MA, et al. Stroke after cardiac surgery: a risk factor analysis of 16,184 consecutive adult patients. Ann Thorac Surg 2003;75:472–8.
17. Likosky DS, Marrin CA, Caplan LR, et al. Determination of etiologic mechanisms of strokes secondary to coronary artery bypass graft surgery. Stroke 2003;34:2830–4.
18. Hogue CW, Murphy SF, Schechtman KB, Davila-Roman VG. Risk factors for early or delayed stroke after cardiac surgery. Circulation 1999;100:642–7.
19. Kimura T, Morimoto T, Furukawa Y, et al. Long-term outcomes of coronary artery bypass graft surgery versus percutaneous coronary intervention for multivessel coronary artery disease in the bare-metal stent era. Circulation 2008; 118(Suppl):199–209.
20. van der Linden J, Casimir-Ahn H. When do cerebral emboli appear during open heart operations? A transcranial Doppler study Ann Thorac Surg 1991;51:237–41.
21. Blauth CI. Macroemboli and microemboli during cardiopulmonary bypass. Ann Thorac Surg 1995;59:1300–3.
22. Barbut D, Yao FS, Lo YW, et al. Determination of size of aortic emboli and embolic load during coronary artery bypass grafting. Ann Thorac Surg 1997;63:1262–7.
23. Bowles BJ, Lee JD, Dang CR, et al. Coronary artery bypass performed without the use of cardiopulmonary bypass is associated with reduced cerebral microemboli and improved clinical results. Chest 2001;119:25–30.
24. Limburg M, Wijdicks EF, Li H. Ischemic stroke after surgical procedures: clinical features, neuroimaging, and risk factors. Neurology 1998;50:895–901.
25. Restrepo L, Wityk RJ, Grega MA, et al. Diffusion- and perfusion-weighted magnetic resonance imaging of the brain before and after coronary artery bypass grafting surgery. Stroke 2002;33:2909–15.
26. van Wermeskerken GK, Lardenoye JW, Hill SE, et al. Intraoperative physiologic variables and outcome in cardiac surgery: part II. Neurologic outcome. Ann Thorac Surg 2000;69:1077–83.
27. Charlesworth DC, Likosky DS, Marrin CA, et al. Development and validation of a prediction model for strokes after coronary artery bypass grafting. Ann Thorac Surg 2003;76:436–43.
28. Lahtinen J, Biancari F, Salmela E, et al. Postoperative atrial fibrillation is a major cause of stroke after on-pump coronary artery bypass surgery. Ann Thorac Surg 2004;77:1241–4.
29. Epstein AE, Alexander JC, Gutterman DD, Maisel W, Wharton JM. Anticoagulation: American College of Chest Physicians guidelines for the prevention and management of postoperative atrial fibrillation after cardiac surgery. Chest 2005;128(Suppl):24–7.
30. Crystal E, Garfinkle MS, Connolly SS, Ginger TT, Sleik K, Yusuf SS. Interventions for preventing post-operative atrial fibrillation in patients undergoing heart surgery. Cochrane Database Syst Rev 2004:CD003611.
31. Hammon JW, Stump DA, Butterworth JF, et al. Coronary artery bypass grafting with single cross-clamp results in fewer persistent neuropsychological deficits than multiple clamp or off-pump coronary artery bypass grafting. Ann Thorac Surg 2007;84:1174–9.

Appendix

List of Participating Centers and Investigators

Centers	Investigators
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Kishiwada City Hospital	Masahiko Onoe
Tenri Hospital	Kazuo Yamanaka
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Hyogo Prefectural Amagasaki Hospital	Shinichi Nomoto
Kokura Memorial Hospital	Hitoshi Okabayashi
Maizuru Kyosai Hospital	Teruaki Ushijima
Nara Hospital, Kinki University School of Medicine	Noboru Nishiwaki
Kobe City Medical Center General Hospital	Yukikatsu Okada
Osaka Red Cross Hospital	Kazuaki Minami
University of Fukui Hospital	Kuniyoshi Tanaka
Shizuoka City Shizuoka Hospital	Mitsuomi Shimamoto
Hamamatsu Rosai Hospital	Masaaki Takahash
Shiga University of Medical Science Hospital	Tohru Asai
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Effect of Intensive Statin Therapy on Regression of Coronary Atherosclerosis in Patients With Acute Coronary Syndrome

A Multicenter Randomized Trial Evaluated by Volumetric Intravascular Ultrasound Using Pitavastatin Versus Atorvastatin (JAPAN-ACS [Japan Assessment of Pitavastatin and Atorvastatin in Acute Coronary Syndrome] Study)

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Objectives The objective of this study was to evaluate whether the regressive effects of aggressive lipid-lowering therapy with atorvastatin on coronary plaque volume (PV) in patients with acute coronary syndrome (ACS) are generalized for other statins in multicenter setting.

Background A previous single-center study reported beneficial regressive effects of atorvastatin in patients with ACS on PV of the nonculprit site by intravascular ultrasound (IVUS) evaluation. The effect of statins other than atorvastatin on PV has not been evaluated in the setting of ACS.

Methods The JAPAN-ACS (Japan Assessment of Pitavastatin and Atorvastatin in Acute Coronary Syndrome) study was a prospective, randomized, open-label, parallel group study with blind end point evaluation conducted at 33 centers in Japan. A total of 307 patients with ACS undergoing IVUS-guided percutaneous coronary intervention were randomized, and 252 patients had evaluable IVUS examinations at baseline and 8 to 12 months' follow-up. Patients were randomly assigned to receive either 4 mg/day of pitavastatin or 20 mg/day of atorvastatin. The primary end point was the percentage change in nonculprit coronary PV.

Results The mean percentage change in PV was −16.9 ± 13.9% and −18.1 ± 14.2% ($p = 0.5$) in the pitavastatin and atorvastatin groups, respectively, which was associated with negative vessel remodeling. The upper limit of 95% confidence interval of the mean difference in percentage change in PV between the 2 groups (1.11%, 95% confidence interval: −2.27 to 4.48) did not exceed the pre-defined noninferiority margin of 5%.

Conclusions The administration of pitavastatin or atorvastatin in patients with ACS equivalently resulted in significant regression of coronary PV (Japan Assessment of Pitavastatin and Atorvastatin in Acute Coronary Syndrome; NCT00242944).
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Abbreviations and Acronyms

ACS = acute coronary syndrome
CRP = C-reactive protein
EEM = external elastic membrane
FAS = full analysis set
HDL-C = high-density lipoprotein cholesterol
IVUS = intravascular ultrasound
LDL-C = low-density lipoprotein cholesterol
PCI = percutaneous coronary intervention
PV = plaque volume

Many large-scale pivotal clinical trials (1–3) have shown that 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitors (statins) reduce both atherogenic lipoproteins as well as cardiovascular morbidity and mortality. In addition, several previous multicenter studies in which the authors used intravascular ultrasound (IVUS) imaging revealed that statins attenuate the progression of atherosclerosis or even diminish plaque volume (4,5). An IVUS study in patients with acute coronary syndrome (ACS) demonstrated that statin therapy with 20 mg/day of atorvastatin could reduce nonculprit coronary plaque volume (6). However, this study was a relatively small trial conducted at a single center. This observation, if confirmed in a larger multicenter study, could address one of the mechanisms of improvement of clinical outcome provided by administration of statins in patients with ACS (7–10).

The effect of statins other than atorvastatin on plaque volume (PV) has not been evaluated in the setting of ACS. Pitavastatin is a statin that is commonly used in Japan, South Korea, and Thailand. It has been demonstrated that its ability to lower levels of low-density lipoprotein cholesterol (LDL-C) is comparable with that of atorvastatin (11). Therefore, a multicenter study using a central IVUS core laboratory was designed to assess the effect of pitavastatin on coronary PV compared with that of atorvastatin in patients with ACS.

Methods

Study design and ethical considerations. The JAPAN-ACS (Japan Assessment of Pitavastatin and Atorvastatin in Acute Coronary Syndrome) study was a prospective, randomized, open-label, parallel group study with blind endpoint evaluation at 33 centers to examine the effect of 8 to 12 months' treatment with pitavastatin versus atrovastatin in coronary plaque regression in nonpercutaneous coronary intervention (PCI) sites of the culprit vessel in patients with ACS. A documentation of the present study design was published before the dataset was locked (12). This study was conducted according to the Declaration of Helsinki and with the approval of the institutional review boards of all 33 participating institutions. Written informed consent to participate was obtained from all of the patients enrolled.

Patient enrollment and randomization. Patients with ACS who satisfied all criteria for inclusion were selected after having a successful PCI under IVUS guidance. We defined ACS as unstable angina pectoris, non–ST-segment elevation myocardial infarction (MI) or ST-segment elevation MI. These diagnoses were made if patients met at least 2 of the following 3 conditions: 1) ischemic ECG changes; 2) the increase (≥2 times) in serum creatine kinase or creatine kinase, myocardial band, or a positive troponin T result; and 3) the presence of symptoms suggestive of ACS. A standard antiplatelet therapy and other medications for ACS were provided.

The patients were randomized within 72 h after PCI to receive either pitavastatin (4 mg) or atorvastatin (20 mg) daily. The dose of 20 mg/day of atorvastatin was selected because when such doses were used in the ESTABLISH (Demonstration of the Beneficial Effect on Atherosclerotic Lesions by Serial Volumetric Intravascular Ultrasound Analysis During Half a Year After Coronary Event) study they significantly reduced coronary PV in patients with ACS (6) and because this dose was the most intensive permitted one to reduce LDL-C in Japan at the beginning of this trial. In addition, the pitavastatin dosage of 4 mg/day was selected because it causes a similar LDL-C–lowering effect to 20 mg/day of atorvastatin (11). We did not include a control group of patients not receiving statin treatment because of ethical reasons. The randomization was stratified by the presence of diabetes mellitus, sex, and total cholesterol level by use of a web response system, which generated association sequence. The IVUS examination was performed at baseline and repeated after 8 to 12 months' administration of the allocated drugs.

Blood examinations for lipid levels and inflammatory markers were performed at baseline and follow-up at 8 to 12 months. Lipid profiles and other biomarkers were measured at SRL Co., Ltd., Tokyo, Japan, and pentraxin3 at Perseus Proteomics Inc., Tokyo, Japan. Safety was evaluated by regular medical examination and laboratory tests at 1, 3, and 8 to 12 months after enrollment. The independent event assessment committee evaluated major adverse cardiac events and any other adverse events.

Examination with IVUS. After IVUS-guided PCI of the culprit lesion of ACS, IVUS examination was performed in both the longest and least angulated culprit vessel segment meeting inclusion criteria. After 200 μg of intracoronary nitroglycerin was administered, a 40-MHz, 2.6-F (0.87-mm) IVUS catheter (Atlantis SR Pro2, Boston Scientific, Natick, Massachusetts) was advanced into the culprit vessel,

honoraria for lectures from Kowa Pharmaceutical, Pfizer, and Astellas Pharma. Dr. Yamagishi has received honoraria for lectures from Kowa Pharmaceutical, Pfizer, and Astellas Pharma and has received a research grant from Kowa Pharmaceutical and Astellas Pharma. Dr. Ozaki is an advisory member of Kowa Pharmaceutical and has received honoraria for lectures from Pfizer and Kowa Pharmaceutical. Dr. Kimura is an advisory member of Kowa Pharmaceutical and has received honoraria for the lectures from Kowa Pharmaceutical and Astellas Pharma. Dr. Saito has received honoraria for lectures from Kowa Pharmaceutical. Dr. Daida is an advisory member of Kowa Pharmaceutical and has received honoraria for lectures and research grants from Kowa Pharmaceutical, Pfizer, and Astellas Pharma. Dr. Matsuzaki is an advisory member of Kowa Pharmaceutical and Pfizer and has received honoraria for lectures and research grants from Kowa Pharmaceutical, Pfizer, and Astellas Pharma.

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and the transducer was positioned as far distally as could be safely reached. This procedure was designed to select the longest-possible vessel segment for analysis. A motorized pullback device withdrew the transducer at a speed of 0.5 mm/s. The consoles used were ClearView or Galaxy 2 systems (Boston Scientific). The same imaging system with the same type of IVUS catheter was used for both the baseline and the follow-up examinations When the angular span of the acoustic shadow of calcification or attenuation by some noncalcified tissues was >90°, the case was excluded. After an 8- to 12-month treatment period, IVUS examinations were performed under the conditions identical to the baseline.

Core laboratory analysis of IVUS. Two independent experienced investigators who were unaware of the patient group allocation performed the quantitative IVUS analysis at the central core laboratory. Baseline and follow-up IVUS images were reviewed together on a display, and target segments were selected. The target segment was determined at a non-PCI site (>5 mm proximal or distal to the PCI site) of culprit vessel with a reproducible index, usually a branch site, on the PCI vessel. Spotty calcification, side vein, and distances from side branch, orifice, left anterior descending-left circumflex branch bifurcation, and stent edge also were referred. Subsequently, every 6th image (0.1 mm apart) was manually traced on a commercially available IVUS measurement software (echoPlaque2, INDEC systems Inc., Santa Clara, California). Moreover, this software automatically interpolated the tracing of 5 cross sections in between the 2 manually traced images. Therefore, the volume was calculated from each of 0.017-mm spaced segments. The final cross section for measurement was obtained at a proximal fiduciary site.

The IVUS measurements were performed according to the standards of the American College of Cardiology and the European Society of Cardiology (13). These measurements are present in the standard manner, for which accuracy and reproducibility have been well established (14). The primary end point was the percent change in coronary PV during the observation period:

$$\frac{\text{PV (follow-up)} - \text{(baseline)}}{\text{PV (baseline)}} \times 100$$

Coronary PV was calculated as the sum of the differences between the external elastic membrane (EEM) and lumen area across all evaluated frames as: $PV = \Sigma(EEM_{CSA} - LUMEN_{CSA})$, where EEM_{CSA} = external elastic membrane cross-sectional area and $LUMEN_{CSA}$ = luminal cross-sectional area.

Major secondary end points include nominal change in percent PV (%PV) and nominal change in normalized plaque volume (NPV) (follow-up minus baseline, respectively):

$$NPV = PV \times \frac{L_{MED}}{L_{MEASURED}}$$

where L_{MED} = median value of observed length in all subjects and $L_{MEASURED}$ = observed length of each plaque.

Statistical analysis. A detailed structure of statistical analyses in the current study was described elsewhere (12). In brief, this study aims to evaluate whether the effect of pitavastatin on coronary PV would not be inferior to that of atorvastatin and vice versa. Two-sided noninferiority was evaluated by analysis of variance with adjustment for sex, the presence of diabetes mellitus, and total cholesterol level on admission as previously described (12). We decided noninferiority margin as follows: 1) in the ESTABLISH study, the % change in PV of atorvastatin was 13.1 ± 12.8%; 2) standard deviation (±12.8) multiplied by 0.36 (15) yielded 4.608, rounded to 5; and 3) the noninferiority margin was decided as 5%. We calculated 150 subjects in each group with an alpha level of 5%, a power of 80%, and a dropout rate of 30%.

We used full analysis set (FAS) of data for primary analyses. Data of patients were included in FAS if patients had ACS and measurable IVUS both at the enrollment and at follow-up. We prepared per-protocol analysis set of data if enrolled patients completely met the inclusion and exclusion criteria and followed the protocol as it was. If patients received the study drug at least once, they were included in the safety analysis set of data.

After the descriptive statistics, comparisons of continuous variables between the 2 groups were performed by the 2-sample *t* test or Wilcoxon rank sum test, and those between the baseline and the follow-up by 1-sample *t* tests or Wilcoxon sign rank test according to their distributions. Comparisons of categorical values between the 2 groups were performed by chi-square tests and Fisher exact tests. We used general linear models to assess relationships between the percent change in coronary PV and several factors, including serum lipid profile at 8 to 12 months, or to assess interobserver and intraobserver variabilities for measuring plaque area. The numbers of adverse events were assessed to determine safety profiles. The significance level was 5% 2-sided (2.5% 1-sided), and all statistical analyses were performed by the use of the SAS system version 9.1 (SAS Institute, Cary, North Carolina).

Results

Patient population. The grouping of patients in the present study is shown in Figure 1. Between November 1, 2005, and October 31, 2006, 307 patients were enrolled at 33 centers in Japan, and 153 patients were randomly assigned to receive pitavastatin and 154 to atorvastatin. The IVUS images qualified for evaluation both at baseline and at follow-up were obtained in 125 patients (82%) in the pitavastatin group and in 127 patients (82%) in the atorvastatin group. The median follow-up time with intraquartile

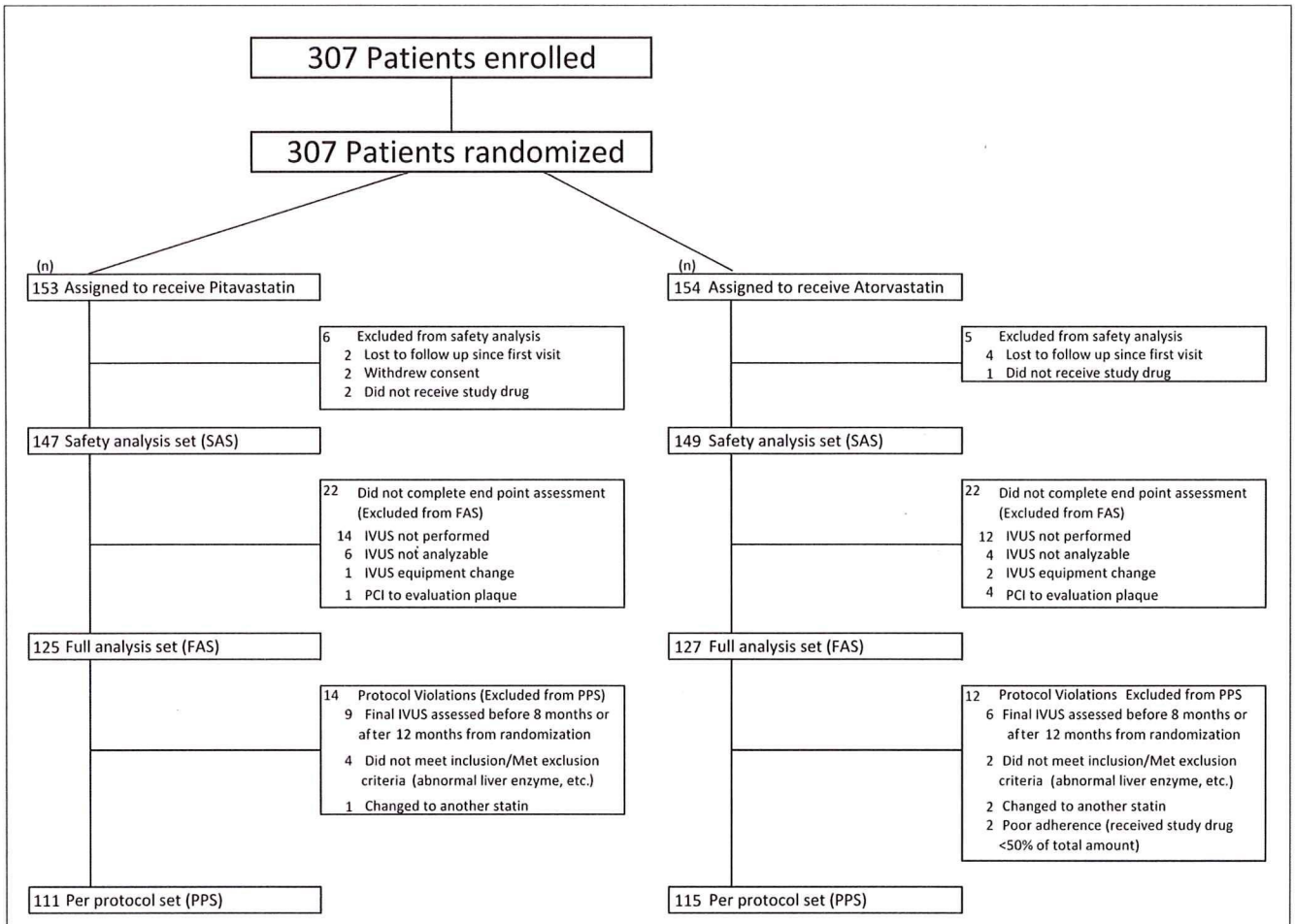

Figure 1 **Disposition of Patients**

FAS = full analysis set; IVUS = intravascular ultrasound; PCI = percutaneous coronary intervention; PPS = per protocol set; SAS = safety analysis set.

range in the pitavastatin group was 9.3 (range 8.5 to 10.3) months and 9.6 (range 8.6 to 10.5) months in the atorvastatin group, respectively.

There was no significant difference in baseline demographics and characteristics between the 2 groups (Table 1). Eighty-two percent of patients were men, and 29% of total patients had diabetes. Sixty-four percent of patients had ST-segment elevation MI, and drug-eluting stents were used in 32% and bare-metal stents in 66%. Plaques proximal to the PCI sites were analyzed in 70% of patients.

Laboratory results. We found that LDL-C decreased from 130.9 ± 33.3 mg/dl (3.39 ± 0.86 mmol/l) at baseline to 81.1 ± 23.4 mg/dl (2.10 ± 0.61 mmol/l) at 8 to 12 months' follow-up ($p < 0.001$) in the pitavastatin group and from 133.8 ± 31.4 mg/dl (3.47 ± 0.81 mmol/l) to 84.1 ± 27.4 mg/dl (2.18 ± 0.71 mmol/l; $p < 0.001$) in the atorvastatin group (Table 2). We found that high-density lipoprotein cholesterol (HDL-C) as well as triglycerides showed comparable increase between the 2 groups. The inflammatory markers, high-sensitivity C-reactive protein, pentraxin3, and white blood cell counts were markedly increased at baseline and were not different between the 2 groups in terms of percent change.

Efficacy analysis with IVUS. We randomly selected 93 IVUS cross-sectional images from 31 patients to assess the intraobserver and interobserver variabilities for measuring plaque area by 2 independent technicians. The correlation coefficient and mean difference ± SD were 0.99 and 0.02 ± 0.24 mm^2 (of the absolute mean value, 6.97 ± 4.33 mm^2, of the samples) for intraobserver variability and 0.98 and 0.13 ± 0.32 mm^2 for interobserver variability.

As a primary end point, the percent change in coronary PV showed a significant regression for both groups (−16.9 ± 13.9% in the pitavastatin group, −18.1 ± 14.2% in the atorvastatin group, and −17.5 ± 14.0% for total patients) (Table 3). Noninferiority of pitavastatin to atorvastatin and also atorvastatin to pitavastatin in terms of percent change in PV was proved (Fig. 2). The mean difference of drug effects on percent change in PV ($\mu p - \mu a$), adjusted for sex, the presence of diabetes mellitus, and total cholesterol level, was 1.11% (95% confidence interval [CI]: −2.27% to 4.48%). The upper limit of 95% CI of this

Table 1 Baseline Patient Characteristics and Concomitant Drugs

Characteristic	Pitavastatin (n = 125)	Atorvastatin (n = 127)
Age (yrs)	62.5 ± 11.5	62.4 ± 10.6
Male	103 (82.4)	103 (81.1)
BMI (kg/m^2)	24.5 ± 3.7	24.2 ± 3.3
Waist circumference (cm)	87.2 ± 9.5	87.0 ± 8.6
Diabetes	36 (28.8)	38 (29.9)
Hypertension	73 (58.4)	84 (66.1)
Family history of CAD	24 (19.2)	21 (16.5)
Smoking	57 (45.6)	62 (48.8)
Alcohol drinker	59 (47.2)	62 (48.8)
Type of ACS		
STEMI	75 (60.0)	87 (68.5)
NSTEMI	18 (14.4)	18 (14.2)
UAP	32 (25.6)	22 (17.3)
Abnormal Q-wave	46 (36.8)	40 (31.5)
Max CK (IU/l), median (IQR)	1,173 (206–2,664)	1,400 (349–2,806)
Culprit vessel		
RCA	33 (26.4)	48 (37.8)
LAD	75 (60.0)	61 (48.0)
LCx	16 (12.8)	18 (14.2)
LMT	1 (0.8)	0
Analysis segment		
Proximal to the treated site	86 (68.8)	90 (70.9)
Disital to the treated site	39 (31.2)	37 (29.1)
BMS	77 (61.6)	89 (70.1)
DES	45 (36.0)	35 (27.6)
Other than stent (POBA)	3 (2.4)	3 (2.4)
Concomitant drugs		
Aspirin	124 (99.2)	124 (97.6)
Ticlopidine	102 (81.6)	107 (84.3)
Clopidogrel	9 (7.2)	8 (6.3)
Beta-blocker	55 (44.0)	61 (48.0)
ACE inhibitor	35 (28.0)	39 (30.7)
ARB	57 (45.6)	68 (53.5)
PPAR-γ agonist	4 (3.2)	6 (4.7)
Sulfonylurea	12 (9.6)	8 (6.3)
α-GI	10 (8.0)	11 (8.7)
Calcium blocker	25 (20.0)	24 (18.9)
Nitrate	21 (16.8)	17 (13.4)
Diuretic	10 (8.0)	9 (7.1)
Aldosterone blocker	3 (2.4)	2 (1.6)
Digitalis	3 (2.4)	2 (1.6)
Other antiplatelet agents	10 (8.0)	7 (5.5)
Warfarin	5 (4.0)	2 (1.6)
Antiarrhythmic agent	1 (0.8)	1 (0.8)

Data are expressed as n (%) unless otherwise specified. Continuous variables were represented by mean ± SD or median (IQR). There were no significant differences of any characteristics between the 2 groups.

ACE = angiotensin-converting enzyme; ACS = acute coronary syndrome; ARB = angiotensin receptor blocker; α-GI = alpha-glucosidase inhibitor; BMI = body mass index; BMS = bare-metal stent; CAD = coronary artery disease; CK = creatine kinase; DES = drug-eluting stent; IQR = intraquartile range; LAD = left anterior descending; LCx = left circumflex branch; LMT = left main trunk; NSTEMI = non–ST-elevation myocardial infarction; POBA = plain old balloon angioplasty; RCA = right coronary artery; STEMI = ST-elevation myocardial infarction; UAP = unstable angina pectoris.

difference did not exceed the pre-defined noninferiority margin of 5%. The direction of difference in per-protocol analysis set setting, mean value of 1.36% (95% CI: −2.15% to 4.88%), was consistent with FAS setting. Secondary efficacy end points such as %PV and normalized PV were significantly reduced in both groups (Table 3).

These benefits were associated with significant negative vessel remodeling in both groups (113.0 ± 59.3 mm^3 to 105.4 ± 55.0 mm^3), which consequently provided slight but significant lumen enlargement (56.1 ± 59.3 mm^3 to 57.8 ± 30.5 mm^3). Reduction in EEM volume correlated with the decreased PV (r = 0.7), but there was no correlation between change in lumen volume and change in PV (Fig. 3). Figure 4 showed representative examples of IVUS in a single patient with ACS at the baseline and the follow-up period in pitavastatin group.

Plaque regression and biomarkers. Because there was no significant difference in percent change in PV between the 2 groups, the correlation between LDL-C level and percent change in PV was evaluated in the whole FAS patients. There were no significant correlations between LDL-C level at follow-up or at baseline and percent change in PV. Percent change in LDL-C level during the study period also did not significant correlate with percent change in PV (Fig. 5). In addition, there were no significant correlations between high-sensitivity C-reactive protein level at follow-up or at baseline and percent change in PV.

Adverse events. There were no significant differences in the prevalence of these major adverse cardiac events and adverse events between the pitavastatin group and the atorvastatin group (Table 4). The study drugs were discontinued because of either adverse reactions or abnormality of laboratory value only in 2.7% and 4.7% of the pitavastatin group and the atorvastatin group, respectively.

Discussion

Our study demonstrated that aggressive lipid-lowering therapy with either pitavastatin 4 mg/day or atorvastatin 20 mg/day achieved significant regression of the coronary PV with negative vessel remodeling in patients with ACS based on a randomized, large-scale, multicenter, central IVUS core laboratory evaluation study. Therefore, the results provided support to the hypothesis that administration of statins after the onset of ACS has the potential to reverse the process of atherosclerosis, thereby improving clinical outcome (7–10). Moreover, the results showed that pitavastatin as well as atorvastatin provided a comparable benefit to reduce PV in such patients. This observation also generalized the effect of statins other than atorvastatin on PV in the setting of ACS.

The degree of percent change in PV was −17.5% for total patients in this study. This beneficial regressive effect was similar to that reported by the ESTABLISH single-center study (−13.1%) (6), even more than that of the REVERSAL trial (−0.4%, median in the atorvastatin group) that used a similar primary end point. One of the potential reasons for this might be the difference in clinical presentation (ACS vs. stable coronary artery disease). Evidence has accumulated that shows

Table 2 Laboratory Results

	Baseline		Follow-Up		Percent Change (%)				
	Pitavastatin (n = 125)	Atorvastatin (n = 127)	Pitavastatin (n = 125)	Atorvastatin (n = 127)	Pitavastatin (n = 125)	p Value Compared With Baseline	Atorvastatin (n = 127)	p Value Compared With Baseline	p Value Between Groups
TC	196.5 ± 35.6	197.9 ± 36.4	151.3 ± 28.0	152.8 ± 33.1	−21.6 ± 16.0	<0.001	−21.9 ± 17.7	<0.001	0.9
LDL-C	130.9 ± 33.3	133.8 ± 31.4	81.1 ± 23.4	84.1 ± 27.4	−36.2 ± 19.5	<0.001	−35.8 ± 22.9	<0.001	0.9
TG	119.2 ± 53.2	116.7 ± 58.1	126.8 ± 80.3	120.7 ± 59.1	16.2 ± 59.9	0.003	21.2 ± 75.5	0.002	0.6
HDL-C	45.0 ± 10.1	43.9 ± 9.4	48.8 ± 12.7	47.1 ± 11.7	9.9 ± 23.5	<0.001	8.0 ± 21.4	<0.001	0.5
HDL-C baseline <40 mg/dl	34.6 ± 3.4	34.5 ± 4.0	41.4 ± 7.2	37.8 ± 7.5	20.6 ± 24.6 (n = 39)	<0.001	10.8 ± 25.4 (n = 46)	0.006	0.08
HDL_2-C	30.4 ± 10.0	29.5 ± 8.6	33.0 ± 12.3	31.7 ± 10.5	10.8 ± 31.4	<0.001	8.7 ± 28.0	<0.001	0.6
HDL_3-C	18.5 ± 3.6	18.0 ± 3.8	17.9 ± 3.2	17.4 ± 3.5	−0.5 ± 21.9	0.8	−1.4 ± 21.5	0.5	0.7
RLP-C	4.5 ± 2.7	4.2 ± 2.4	4.1 ± 3.6	3.7 ± 2.5	4.7 ± 87.9	0.6	6.2 ± 80.9	0.4	0.9
Small dense LDL (RM value)	0.35 ± 0.04	0.36 ± 0.05	0.34 ± 0.04	0.34 ± 0.03	−2.6 ± 11.1	0.01	−3.4 ± 11.9	0.002	0.6
Non-HDL-C	151.1 ± 33.1	153.5 ± 33.7	102.6 ± 25.2	105.7 ± 32.2	−30.5 ± 18.9	<0.001	−30.1 ± 20.8	<0.001	0.9
LDL-C/HDL-C	3.0 ± 0.9	3.2 ± 0.9	1.8 ± 0.6	1.9 ± 0.7	−40.3 ± 19.4	<0.001	−38.8 ± 23.1	<0.001	0.6
ApoA-I	111.8 ± 19.8	109.8 ± 19.2	130.9 ± 24.8	124.5 ± 24.1	18.5 ± 21.6	<0.001	14.0 ± 19.4	<0.001	0.1
ApoB	103.6 ± 23.3	104.8 ± 23.2	73.3 ± 17.0	74.6 ± 21.3	−27.6 ± 18.3	<0.001	−27.6 ± 20.2	<0.001	0.99
ApoE	4.1 ± 1.2	4.2 ± 1.1	3.6 ± 1.0	3.5 ± 1.0	−9.4 ± 23.5	<0.001	−12.7 ± 23.5	<0.001	0.3
ApoB/ApoA-I	0.95 ± 0.25	0.97 ± 0.24	0.58 ± 0.16	0.62 ± 0.22	−37.7 ± 16.1	<0.001	−35.7 ± 18.2	<0.001	0.4
MDA-LDL (U/l)	130.2 ± 43.6	128.9 ± 49.1	88.1 ± 27.4	93.9 ± 34.6	−28.4 ± 25.3	<0.001	−22.1 ± 28.2	<0.001	0.1
Phospholipid	200.4 ± 30.8	199.0 ± 33.1	183.7 ± 31.1	177.4 ± 29.8	−7.4 ± 15.8	<0.001	−10.1 ± 14.4	<0.001	0.2
Lp(a)	20.2 ± 16.7	22.9 ± 21.9	22.7 ± 22.0	24.2 ± 25.8	5.9 ± 45.6	0.2	9.8 ± 54.9	0.049	0.5
hs-CRP (mg/l), median (IQR)	19.9 (7.1–71.1)	14.9 (4.7–62.4)	0.54 (0.36–1.1)	0.53 (0.26–1.2)	−97.3 (−99.1 to −89.6)	<0.001*	−95.4 (−99.0 to −84.5)	<0.001*	0.4†
PTX3 (ng/ml), median (IQR)	5.9 (3.8–8.9)	5.6 (3.8–9.0)	2.1 (1.5–2.8)	1.9 (1.3–2.6)	−64.3 (−77.5 to −45.1)	<0.001*	−68.9 (−79.1 to −52.4)	<0.001*	0.9†
WBC (cells/l), median (IQR)	8,820 (7,015–11,400)	9,300 (7,580–11,200)	6,000 (5,300–7,200)	6,000 (5,005–7,268)	−31.6 (−44.9 to −16.6)	<0.001*	−33.6 (−45.1 to −17.2)	<0.001*	0.7†
HbA1c (%)	5.9 ± 1.3	6.0 ± 1.1	5.9 ± 1.1	5.9 ± 0.89	0.6 ± 10.5	0.5	0.6 ± 10.5	0.5	0.98

Values are mg/dl unless otherwise indicated. Continuous variables were represented by mean ± SD or median (IQR). The last column indicates the comparison of percent change in valuables between pitavastatin and atorvastatin group. SI conversions: To convert total cholesterol, LDL-C, HDL-C, HDL_2-C, HDL_3-C, remnant lipoprotein-C (RLP-C), non-HDL-C to mmol/l, multiply by 0.0259; apolipoprotein (apo)A-I, apoB, apoE to g/l, multiply by 10; malonyl-dialdehyde (MDA)-low-density lipoprotein (LDL), phospholipid and lipoprotein (a) [Lp(a)] to mg/l, multiply by 10; PTX3 to μg/l, multiply values by 1. *Wilcoxon sign rank test. †Wilcoxon rank sum test.

CRP = C-reactive protein; Hb = hemoglobin; HDL-C = high-density lipoprotein cholesterol; hs-CRP = high-sensitivity C-reactive protein; IQR = intraquartile range; IVUS = intravascular ultrasound; LDL-C = low-density lipoprotein cholesterol; PCI = percutaneous coronary intervention; PTX3 = pentraxin 3; RM = relative migration; TC = total cholesterol; WBC = white blood cell counts.