Original article

Effect of polymer-free TiO2 stent coated with abciximab or alpha lipoic acid in porcine coronary restenosis model

Kyung Seob Lim (DVM, MS), Myung Ho Jeong (MD, PhD, FACC, FAHA, FESC), In Ho Bae (PhD), Jun-Kyu Park (MS), Dae Sung Park (MS), Jong Min Kim (MS), Jung Ha Kim (BS), Hyung-Seok Kim (MD, PhD), Yong Sook Kim (PhD), Hye-Yun Jeong (BS), Sun-Jung Song (PhD), Eun Ju Yang (MS), Dong Lyun Cho (PhD), Doo Sun Sim (MD, PhD), Keun-Ho Park (MD, PhD), Young Joon Hong (MD, PhD), Youngkeun Ahn (MD, PhD)

Korea Cardiovascular Stent Institute, Jangsung, Republic of Korea
Cardiovascular Convergence Research Center Nominated by Korea Ministry of Health and Welfare, Chonnam National University Hospital, Gwangju, Republic of Korea
Cardiovascular Research Center, Chonnam National University Hospital, Gwangju, Republic of Korea
Regeneromics Research Center, Chonnam National University, Gwangju, Republic of Korea

Article info

Article history:
Received 23 July 2013
Received in revised form 21 January 2014
Accepted 6 February 2014
Available online 19 May 2014

Keywords:
Stents
Percutaneous coronary intervention
Restenosis
Inflammation

Abstract

Background: Polymer-free drug-eluting stents (DES) may overcome the shortcomings of polymer-based DES. The aim of this study was to examine the effect of the polymer-free TiO2 film-coated stent with abciximab or alpha lipoic acid in a porcine coronary overstretch restenosis model.

Methods: Pigs were randomized into four groups in which the coronary arteries (24 pigs, 48 coronaries in each group) had TiO2 film-coated stent with abciximab (TCA, n = 12), TiO2 film-coated stent with alpha lipoic acid (TCALA, n = 12), biolimus A9-eluting stents with biodegradable polymer (BES, n = 12), and TiO2 film-coated stent (TCstent, n = 12). Histopathologic analysis was performed at 28 days after stenting.

Results: There was no significant difference in the injury score and internal elastic lamina (IEL) among the four groups. There were significant differences in the lumen area, neointima area, percent area stenosis, fibrin score, and inflammation score among the four groups [2.7 ± 1.0 mm², 2.9 ± 0.83 mm², 53.5 ± 17.19%, 1.0 (range 0.0–2.0), 1.0 (range 0.0–2.0) in TCA stent group vs. 2.7 ± 1.24 mm², 2.6 ± 0.94 mm², 48.9 ± 16.25%, 1.0 (range 0.0–2.0), 1.0 (range 0.0–2.0) in TCALA stent group vs. 2.7 ± 1.30 mm², 2.6 ± 1.06 mm², 50.1 ± 23.20%, 2.0 (range 1.0–3.0), 1.0 (range 0.0–2.0) in BES group vs. 1.7 ± 0.63 mm², 3.3 ± 0.58 mm², 60.2 ± 10.12%, 0.5 (range 0.0–2.0), 1.0 (range 0.0–2.0) in TC stent group, respectively].

Conclusion: TCA and TCALA are more effective to reduce neointimal hyperplasia compared to TC. Moreover, fibrin and inflammation scores are significantly lower in TCA and TCALA than BES in porcine coronary restenosis model.

© 2014 Published by Elsevier Ltd on behalf of Japanese College of Cardiology.

Introduction

In recent years, drug-eluting stents (DES) have emerged as the preferred treatment for coronary artery disease and the use of DES has increased significantly. Current DES treatment for coronary artery disease is promising, but it has the drawback of late in-stent thrombosis and delayed re-endothelialization [1]. These can be related to polymers, which were used in bare metal stent coating [2–4]. Therefore, we developed a novel and efficient drug-combining technology onto a TiO2 film without the polymers to reduce the problems of the polymer-based DES [5].
Abciximab blocks the final common pathway of platelet aggregation [6]. Abciximab reacts to αVβ3 receptor of vascular smooth muscle cell and to Mac-1 of macrophage and inhibits proliferation of vascular smooth muscle cells and inflammatory reaction [7–9]. Our previous study showed that abciximab-coated stent reduced stent restenosis by inhibition of cell proliferation, inflammation reaction, and extracellular matrix synthesis compared with bare-metal stent in a porcine coronary restenosis model [10,11]. Alpha lipoic acid (ALA) is a potent antioxidant, which has a direct free-radical scavenger effect [12,13]. Moreover, ALA inhibits the inflammatory reaction and suppresses neointima formation after stenting [14]. Our previous study showed that ALA-coated stent reduces in-stent restenosis in a porcine coronary restenosis model [15].

Biolimus A9-eluting stents (BES, Biomatrix, Biosensors Interventional Technologies Pte Ltd., Singapore), which were developed as a third-generation DES, contain a biodegradable polymer with biolimus A9.

The objective of the present study was to evaluate the effect of polymer-free TiO2 film-coated stent with abciximab or ALA in a porcine coronary restenosis model.

Methods

Animal preparation and stent implantation

The animal study was approved by the Ethics Committee of Chonnam National University Medical School and Chonnam National University Hospital (CNU IACUC-H-2012-24), and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Study animals were castrated male pigs weighing 20–25 kg. To prevent acute thrombosis after stenting, premedication with aspirin 100 mg and clopidogrel 75 mg pigs weighing 20–25 kg. To prevent acute thrombosis after stenting, premedication with aspirin 100 mg and clopidogrel 75 mg per day was given for 5 days before the procedure. On the procedure day, pigs were anesthetized with zolazepam and tile-tamine (2.5 mg/kg, Zoletil®50®, Virbac, Caros, France), xylazine (3 mg/kg, Rompun®, Bayer AG, Leverkusen, Germany), and azaperone (6 mg/kg, Stresnil®, Janssen-Cilag, Neuss, Germany). They received supplemental oxygen continuously through an oxygen mask. Subcutaneous 2% lidocaine at the cut-down site was administered, left carotid artery was surgically exposed, and a 7-French sheath was inserted.

Continuous hemodynamic and surface electrocardiographic monitoring were maintained throughout the procedure. Then 5000 units of heparin were administered intravenously as a bolus prior to the procedure, the target coronary artery was engaged using standard 7-F guide catheters and control angiograms of both coronary arteries were performed using nonionic contrast agent in two orthogonal views.

The stent was deployed by inflating the balloon and the resulting stent-to-artery ratio was 1.3:1. Coronary angiograms were obtained immediately after stent implantation. Then, all equipments were removed and the carotid artery was ligated.

Four weeks after stenting, the animals underwent follow-up angiography in the same orthogonal views before death with 20 ml of potassium chloride intracoronary injection.

The hearts were removed, and the coronary arteries were pressure-perfusion fixed at 110 mmHg in 10% neutral buffered formalin overnight. Each of the 10 stented arteries was step-sectioned, processed routinely for light microscopy, and stained for histological analysis in four groups. And each of the 2 specimens of four groups was analyzed by both scanning electron microscopy (SEM) and micro-computerized tomography (M-CT).

Study groups

The pigs were randomly divided into 4 groups: group 1 (TiO2 film-coated stent with abciximab, TCA stent, 3.0 mm × 16 mm, n = 12); group 2 (TiO2 film-coated stent with ALA, TCA/ALA stent, 3.0 mm × 16 mm, n = 12); group 3 (biolimus A9-eluting stents with biodegradable polymer, BES, 3.0 mm × 18 mm, n = 12); and group 4 (TiO2 film-coated stent, TC stent, 3.0 mm × 16 mm, n = 12).

A total of 24 pigs were used in this study (24 pigs, 48 coronary arteries, 12 coronary arteries in each group). A TCA stent, a TCA/ALA stent, a BES, and a TC stent were implanted in the left anterior descending artery and left circumflex artery by randomized manner in a pig.

Preparation of TiO2 film-coated stent with abciximab and ALA

A TiO2 thin film was deposited onto a bare metal stent (3.0 mm × 16 mm, cobalt–chromium alloy) by the plasma-enhanced chemical vapor deposition (PECVD) process and its potential as a drug-combining matrix was investigated. When deposited at a discharge power of 5 W, the film showed a highly smooth surface with a surface roughness of 9.4 nm, mechanical stability with good adhesion, and good blood compatibility. The film was surface-modified with water plasma to introduce hydroxyl groups on the TiO2 surface. Then, drugs could be chemically grafted to the modified surface through the formation of ester bonds between hydroxyl groups on the modified TiO2 film and carboxyl groups in the drugs. When abciximab or ALA was grafted onto the TiO2–deposited and surface-modified stents, the grafted amount was measured to be 106.1 μg for ALA and 32.5 μg for abciximab on average. In the in vitro drug-release test, abciximab was released continuously for 4 weeks but ALA showed a burst release within 6 days [5].

Histopathologic, scanning electron microscopy, micro-computed tomography, and immunohistochemistry analyses

Histopathologic evaluation of each artery was performed by an experienced cardiovascular pathologist. The specimens were embedded and sections of 50–100 μm thickness were obtained at about 1 mm apart and stained with hematoxylin–eosin (Fig. 1), Verhoeff’s Van Gieson (Fig. 2), and Carstairs’ (Fig. 3) for histological analysis. Measurements of the histopathologic sections were performed using a calibrated microscope, digital video imaging system, and microcomputer program (Visus 2000 Visual Image Analysis System, IMT Tech, CA, USA). Borders were manually traced for lumen area, area circumscribed by the internal elastic lamina, and the innermost border of the external elastic lamina (external elastic lamina area). Morphometric analysis of the neointimal area for a given vessel was calculated as the measured internal elastic lamina area minus lumen area. The measurements were made on five cross-sections from the proximal and distal ends and the three midpoints of each stented segment. Histopathologic stenosis was calculated as 100 × [1 – (lesion lumen area/lesion internal elastic lamina area)] [16]. The harvested stent specimen was stored in formaldehyde solution. A 1.5-ml Eppendorf tube was filled with clay, and the clay was turned with a V shape to hold the stent during contrast agent staining. The stents were taken from the solution and placed vertically in the V-shaped opening in the clay. Each stent had to be fixed with the clay such that there was no movement of the stent inside the Eppendorf tube. The contrast agent used was omnipexol. One milliliter of the contrast agent was taken in a 5-ml syringe and injected through the opening at the center of the stent. The stent was incubated with contrast agent overnight and subjected to M-CT imaging [17]. After performing the M-CT analysis, for SEM,
Fig. 1. Representative images of hematoxylin and eosin staining at 4 weeks after stenting. Specimen TCA stent implanted (A: 20×), TCALA stent implanted (B: 20×), BES implanted (C: 20×) and TC stent implanted (D: 20×). Immunohistochemistry using anti-smooth muscle actin monoclonal antibody in neointimal proliferation. Immunofluorescence staining showing expression of α-smooth muscle actin (bright red positive cells, 200×). A-1 = TCA, B-1 = TCALA, C-1 = BES, D-1 = TC. TCA stent, TiO2 film-coated stent with abciximab; TCALA stent, TiO2 film-coated stent with alpha lipoic acid; BES, biolimus A9-eluting stent with biodegradable polymer; TC stent, TiO2 film-coated stent.

(For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the longitudinally cut sections were rinsed in three changes of sodium phosphate, fixed in 1% osmium tetroxide, and rinsed in distilled water. The specimens were dehydrated in a graded series of alcohols, critically point dried, placed in a vacuum coater, and coated with 30–40 nm gold. Luminal surfaces were photographed at 100×. Photographs were digitized, and the strut coverage was observed [18]. Immunohistochemistry (IHC) was conducted by standard procedures as previously described [19]. Monoclonal
anti-actin (alpha-smooth muscle antibody produced in mouse, Sigma–Aldrich, St. Louis, MO, USA) was used. IHC was analyzed by fluorescence microscopy and digital photography. All results were interpreted by two independent pathologists in a blinded fashion.

Evaluation of arterial injury

Arterial injury at each strut site was determined by the anatomic structures penetrated by each strut. A numeric value was assigned, as previously described by Schwartz et al. [16]: 0 = no injury;
Fig. 3. The Carstair fibrin stain of the low- and high-power fields (magnitude, 20×, 200×) of fibrin infiltration in TCA stent implanted (A, A-1), TCALA stent implanted (B, B-1), BES implanted (C, C-1: black arrow indicates fibrin deposition) and TC stent implanted (D, D-1). TCA stent, TiO2 film-coated stent with abciximab; TCALA stent, TiO2 film-coated stent with alpha lipoic acid; BES, biolimus A9-eluting stent with biodegradable polymer; TC stent, TiO2 film-coated stent.

1 = break in the internal elastic membrane; 2 = perforation of the media; 3 = perforation of the external elastic membrane to the adventitia. The average injury score for each segment was calculated by dividing the sum of injury scores by the total number of struts at the examined section.

Evaluation of inflammation scores, neointimal reaction, and fibrin score

With regard to the inflammation score for each individual strut, the grading was as follows: 0 = no inflammatory cells surrounding the strut; 1 = light, noncircumferential lymphohisti-
ocytic infiltrate surrounding strut; 2 = localized, moderate to dense cellular aggregate surrounding the strut noncircumferentially; 3 = circumferential dense lymphohistiocytic cell infiltration of the strut. The inflammation score for each cross section was calculated by dividing the sum of the individual inflammation scores by the total number of struts at the examined section [20]. Ordinal data for fibrin were collected on each stent section using a scale of 0–3 as previously reported [21].

Statistical analysis

Statistical analysis was performed with the aid of the commercially available software (SPSS Version 15, Chicago, IL, USA). The data were presented as mean value ± SD. Unpaired Student’s t test was used for the comparison of each stent group. Analysis of variance (ANOVA) was used for comparisons of the four stent groups. Ordinal measurements such as injury score, fibrin score, and inflammation score were analyzed using the Kruskal–Wallis test. Non-parametric results were presented as median and interquartile range. A value of p < 0.05 was considered statistically significant.

Results

Analysis after stenting

Two stents were placed for two coronary arteries per swine. A total of 48 stents, including 12 TCA stents, 12 TCALA stents, 12 BESs, and 12 TC stents, were placed in the proximal left anterior descending and proximal circumflex artery for 24 swine. Mortality for this study was zero. There was no significant difference in stent-to-artery ratio among the four stent groups.

Histopathologic and immunohistochemistry analyses among 4 groups

To confirm the characteristics of smooth muscle cells (SMCs) in neointima proliferation tissue, the stented arteries were stained by anti-SMC antibody. SMCs were major components of neointima tissue after stenting in all groups (Fig. 1).

There were no significant differences in the injury score [2.0 (range 1.0–3.0) in TCA stent group vs. 2.0 (range 1.0–3.0) in TCALA stent group vs. 2.0 (range 1.0–3.0) in BES group vs. 2.0 (range 1.0–2.0) in TC stent group, p = NS] and in the internal elastic lamina (5.3 ± 0.99 mm² in TCA stent group vs. 5.6 ± 1.05 mm² in TCALA stent group vs. 5.3 ± 0.71 mm² in BES group vs. 5.0 ± 0.68 mm² in TC stent group, p = NS) among the four groups. There were significant differences in the lumen area (2.7 ± 1.0 mm² in TCA stent group vs. 2.7 ± 1.24 mm² in TCALA stent group vs. 2.7 ± 1.30 mm² in TC stent group vs. 2.8 ± 0.83 mm² in TCALA stent group vs. 2.6 ± 1.06 mm² in BES group vs. 3.3 ± 0.58 mm² in TC stent group, p < 0.001), in the percent area stenosis (48.9 ± 16.25% in TCA stent group vs. 53.5 ± 17.19% in TCALA stent group vs. 50.1 ± 23.20% in BES group vs. 60.2 ± 10.12% in TC stent group, p < 0.001), in the circularity score [1.0 (range 0.0–3.0) in TCA stent group vs. 1.0 (range 0.0–2.0) in TCALA stent group vs. 2.0 (range 1.0–3.0) in BES group vs. 1.0 (range 0.0–2.0) in TC stent group, p < 0.0001] among the four groups (Fig. 4).

Scanning electron microscopic analysis

SEM revealed complete stent strut coverage in all groups. There was no significant difference in strut coverage among the four stent groups (Fig. 5).

Micro-computed tomography analysis

In-stent restenosis rate of M-CT showed similar results with percent area stenosis in histopathologic analysis (51.3 ± 15.2% in TCA stent group vs. 51.8 ± 10.6% in TCALA stent group vs. 46.0 ± 12.7% in BES group vs. 68.5 ± 20.1% in TC stent group) (Fig. 6).

Discussion

Our study was conducted to compare the TCA (TiO2 film-coated stent with abciximab) and TCALA (TiO2 film-coated stent with ALA) with BES (biolimus A9-eluting stent with biodegradable polymer) and TC (TiO2 film-coated stent) in a porcine coronary restenosis model. Our study showed that TCA and TCALA appeared to be effective on inhibition of the neointimal proliferation and fibrin and inflammation scores were significantly lower than BES. The results demonstrated that the polymer that induces inflammation and delayed arterial healing was not used in TiO2 film-coated stent; therefore, fibrin and inflammation scores of TCA and TCALA are significantly lower than with a polymer-based drug delivery stent.

To measure the re-endothelialization, the stented coronary tissues should have treated as methyl methacrylate or glycol methacrylate block which is not remove stent strut. However, we treated paraffin block. So, we were unable to measure the re-endothelialization. We conducted IHC using CD31, CD34, and von Willebrand factor for endothelium detection. However, the stent strut extraction eliminated weak single layer endothelium. Therefore, we found very rare fluorescent positive cells in the specimen.

Bare metal stents can cause inflammatory reactions, which lead to neointimal proliferation around the stent struts [22]. Titanium is commonly used in biomedical materials for dental and orthopedic devices because it has good blood compatibility, biochemical stability, and mechanical attributes. TiO2 films have superior blood compatibility compared to stainless steel, gold, and other stent materials [23]. When TiO2 is coated on a bare metal stent, it enhances anticoagulation effect and inhibits inflammatory reaction [24]. So we covered with a TiO2 thin film onto a cobalt–chromium bare metal stent by the (PECVD) process. Then, abciximab and ALA were grafted onto the modified TiO2 film-coated stents through covalent bonding without polymer [5].

Our previous study demonstrated that abciximab-coated stents showed comparable inhibition of inflammatory cell infiltration and neointimal hyperplasia with sirolimus-eluting stents and paclitaxel-eluting stents in a porcine coronary restenosis model [11]. And abciximab-coated stents significantly inhibit neointima hyperplasia in human coronary de novo lesions [10]. Moreover abciximab-coated stents were safe and effective without stent thrombosis in a prospective randomized trial of patients with acute myocardial infarction [25]. The possible mechanisms responsible for inhibition of neointimal hyperplasia by abciximab might be anti-platelet, anti-inflammatory, and anti-proliferative actions.

ALA is a potent anti-oxidant agent and is beneficial for improving endothelial dysfunction [26]. And ALA is an effective agent to reduce inflammatory processes [27]. Moreover, ALA prevents neointimal hyperplasia and late stent thrombosis after angioplasty or DES implantation [14,28]. In our previous study, ALA-coated stents inhibited neointimal hyperplasia in the porcine coronary restenosis model [15]. In our previous study using dual-coated
Fig. 4. Injury score (A), internal elastic lamina (B), lumen area (C), neointima area (D), % area stenosis (E), fibrin score (F) and inflammation score (G), in TCA, TCALA, BES and TC. A, F and G are expressed as median (interquartile range). TCA stent, TiO$_2$ film-coated stent with abciximab; TCALA stent, TiO$_2$ film-coated stent with alpha lipoic acid; BES, biolimus A9-eluting stent with biodegradable polymer; TC stent, TiO$_2$ film-coated stent.
Fig. 5. Scanning electron microscopic analysis of the stent-implanted coronary arteries. TCA stent implanted (A: 100×), TCALA stent implanted (B: 100×), BES implanted (C: 100×) and TC stent implanted (D: 100×). TCA stent, TiO$_2$ film-coated stent with abciximab; TCALA stent, TiO$_2$ film-coated stent with alpha lipoic acid; BES, biolimus A9-eluting stent with biodegradable polymer; TC stent, TiO$_2$ film-coated stent.

Fig. 6. Micro-computed tomography (M-CT) of in-stent restenosis (ISR) with TCA, TCALA, BES, and TC. TCA stent, TiO$_2$ film-coated stent with abciximab; TCALA stent, TiO$_2$ film-coated stent with alpha lipoic acid; BES, biolimus A9-eluting stent with biodegradable polymer; TC stent, TiO$_2$ film-coated stent.
The BES with biodegradable polymer that was developed as a third generation DES elutes new-limus derivative biolimus A9 from a bioabsorbable polyactic acid (PLA) polymer [30]. The polymers are known to be related to restenosis, inflammation, and late stent thrombosis. But BES releases biolimus A9 into the vessel wall while the PLA polymer is decomposed by contacted coronary artery tissues. Therefore, BES with bioabsorbable polymer significantly reduces the risk of very late stent thrombosis compared to DES with durable polymer.

In our previous study using BES, BES appears to be reliable on the inflammatory reaction at overlapping segments as well as non-overlapping segments [31]. In clinical research, BES showed a lower rate of the composite of major adverse cardiac events in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention compared with a BMS [32].

TCA and TCALA showed a similar neointimal suppressive effect compared to commercial BES which used strong immunosuppressive agents such as sirolimus derivatives in this experiment. TC can replace BMS at the present time. We will attempt the clinical application of TCA and TCALA after long-term follow-up experiment using minipigs. In our advanced research, the TC-based-limus derivatives eluting stent (TCL) is under development. We think that developing TCL can replace the conventional DES with polymer.

Study limitations

Our study had some limitations. First, we used normal porcine coronary arteries without atherosclerotic lesions unlike the human clinical situation with pre-existing atherosclerosis. Second, we examined inflammatory reaction based on H&E stain. IHC techniques are the standard for such studies [33]. Third, we did not perform long-term follow-up experiments such as over 6 months using minipigs.

Conclusion

This study shows that TCA and TCALA are more effective to reduce neointimal hyperplasia compared to TC. Moreover, inhibition of neointimal proliferation of TCA and TCALA is not inferior to commercial BES and fibrin and inflammation scores are significantly lower than with BES at 1 month after stenting in a porcine coronary restenosis model.

Disclosures

None.

Acknowledgments

This study was supported by grant of The Korean Health Technology R&D Project (H1131C1527), Ministry of Health & Welfare, Republic of Korea, Cardiovascular Research Center, Chonnam National University Hospital and Regeneromics Research Center, Chonnam National University.

References


