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Basic Science Review

Particle Debris From a Nanoporous Stent Coating Obscures Potential Antiproliferative Effects of Tacrolimus-Eluting Stents in a Porcine Model of Restenosis

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Polymer stent coatings may not be suitable for drug elution because of inherent proinflammatory effects. A previous study suggested a beneficial effect of a stent eluting tacrolimus from a nanoporous ceramic aluminum oxide coating in a rabbit restenosis model. We investigated whether this stent is effective in preventing in-stent restenosis in a porcine restenosis model. Thirty-four juvenile swine underwent balloon overstretch injury and were subjected to implantation of either stainless steel (bare) stents, bare stents coated with nanoporous aluminum oxide alone, and coated stents eluting 50 and 180 μg of tacrolimus (FK506). In-stent restenosis was quantified at 1 and 3 months after stent placement by histomorphometry. A significant increase of neointimal hyperplasia was noted with the stents coated with aluminum oxide alone compared with bare stents (2.92 ± 1.02 and 1.38 ± 0.51 mm^2, respectively; $P < 0.02$). In all arteries containing coated stents, particle debris was found in the media and neointima, resulting in augmented vascular inflammation. In the group of stents coated with aluminum oxide, FK506 elution at a dose 180 μg reduced neointimal hyperplasia vs. no drug elution (1.66 ± 0.49 vs. 2.92 ± 1.02 mm^2; 180 μg vs. ceramic alone; $P < 0.03$). At a dose of 50 μg stent-based delivery of FK506, no reduction of neointimal hyperplasia was found (2.88 ± 1.31 and 2.92 ± 1.02 mm^2, respectively; P = NS; FK506 vs. ceramic alone). In summary, particle debris shed from a drug-eluting aluminum oxide coating of a stainless steel stent counteracts potential antiproliferative effects of stent-based tacrolimus delivery in a porcine model of restenosis. We propose that stent coatings eluting drugs need to be routinely tested for being tightly anchored into the stent surface. Alternatively, omission of any coating used as a drug reservoir may eliminate inflammatory particle debris after placement of drug-eluting stents. *Catheter Cardiovasc Interv 2005;64:85–90.*

Key words: drug-eluting stent; inflammation; tacrolimus; restenosis

INTRODUCTION

The major complication of coronary stenting is in-stent restenosis, which is due to excessive neointimal proliferation and remains a therapeutically challenge [1,2]. Different approaches have been utilized to avoid in-stent restenosis, including endovascular irradiation [3], radioactive stents [4], and drug-eluting stents [5–7]. To date, several agents eluting from stents have been studied, including paclitaxel, QP-2, rapamycin, actinomycin D, dexamethason, tacrolimus, and everolimus. Different studies, published recently or are still ongoing, have evaluated these drugs with respect to their release kinetics, effective dosage, safety in clinical practice, and

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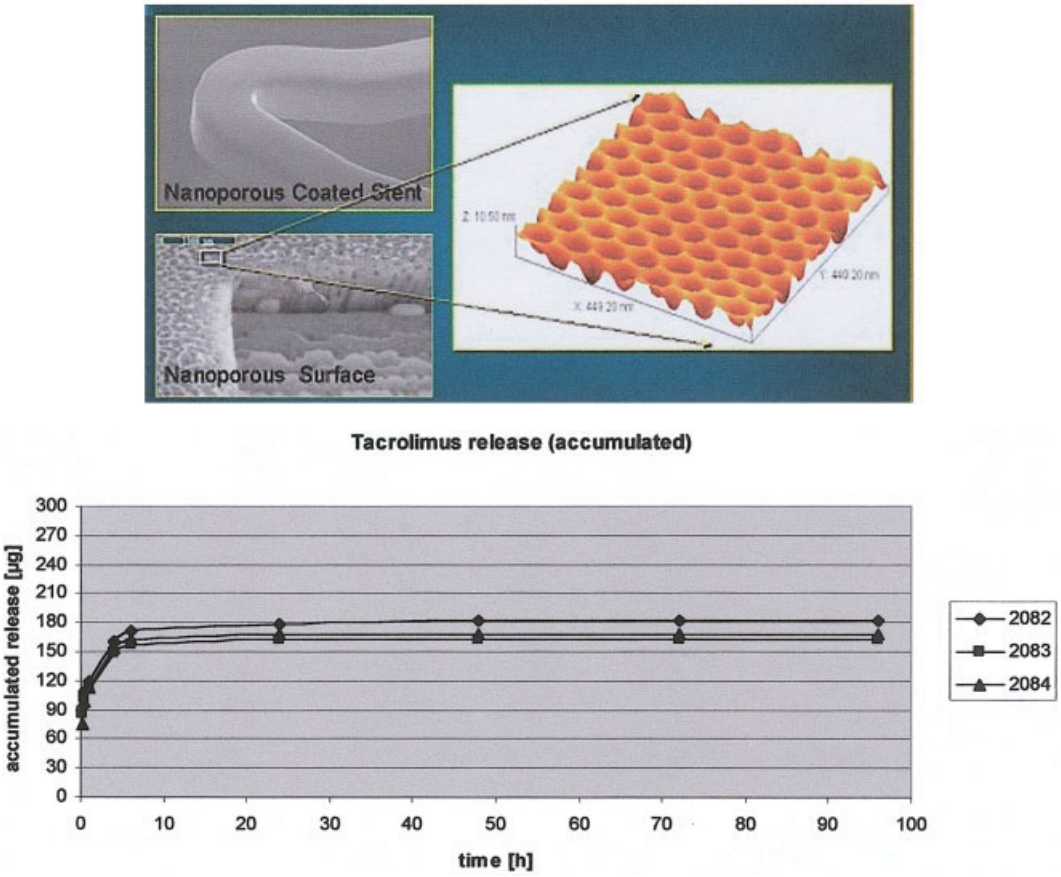

Fig. 1. A: Nanoporous stent coating using aluminum oxide ceramic material. B: Tacrolimus release from a nanoporous aluminum oxide stent coating. Lot 2082 initial loading, 210 µg/stent; lot 2083 initial loading, 216 µg/stent; lot 2084 initial loading, 231 µg/stent. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

benefit [8–12]. Sirolimus (rapamycin) is a potent inhibitor of smooth muscle cell (SMC) proliferation and migration. Rapamycin-mediated inhibition of SMC proliferation is associated with upregulation of the cyclin-dependent kinase inhibitor p27 (Kip1) [13]. Tacrolimus (FK506) is a macrolide antibiotic with immunosuppressive properties. This agent may enhance expression of the transforming growth factor β1 gene and was suggested to inhibit T-cell proliferation in response to upregulation of the T-cell receptor [14].

Stent coatings made from polymers have caused substantial local inflammatory response in the past, and even today refinement of the polymer coating technology leaves room for improvements with respect to biocompatibility [15]. Alternatively, nonpolymeric coatings may reduce potential adverse effects. A recently published previous study found a positive synergistic effect of a nanoporous ceramic stent coating combined with the elution of tacrolimus in a rabbit restenosis model [16].

The present study was designed and conducted according to the recommendations of the American Consensus Group for Research on drug-eluting stents [17] to evaluate this novel stent in a porcine coronary restenosis model. The nanoporous ceramic stent coatings were manufactured as described previously [16] and were loaded with two potentially effective doses of tacrolimus (FK506) to inhibit in-stent neointimal hyperplasia.

MATERIALS AND METHODS

Model of Porcine Coronary Stent Overstretch Injury

Thirty-four domestic juvenile swine, 35–40 kg in weight, were implanted with either bare stents, stents with nanoporous aluminum oxide coating, 50 µg or 180 µg tacrolimus eluting from a nanoporous aluminum oxide-coated stents (JOMED, Rangendingen, Germany) in the LAD and LCX or RCA. Stents were

TABLE I. Histomorphometric Analysis 4 and 12 Weeks After Implantation of Either Stainless Steel, Ceramic Coated, or Tacrolimus-Eluting Ceramic-Coated Stents

	Stainless steel	Ceramic	50 μg tacrolimus	180 μg tacrolimus
4 weeks	**n = 12**	**n = 9**	**n = 6**	**n = 6**
Neointimal thickness (mm)	0.15 ± 0.07	0.41 ± 0.19[a]	0.32 ± 0.17[a]	0.19 ± 0.08[b]
Neointima area (mm^2)	1.38 ± 0.51	2.92 ± 1.02[a]	2.88 ± 1.31[a]	1.66 ± 0.49[b]
% stenosis	26.2 ± 9.7	52.1 ± 1.9%[a]	41.8 ± 16.9[a]	30.1 ± 8.6[b]
Injury score	0.19 ± 0.18	0.49 ± 0.60	0.35 ± 0.26	0.22 ± 0.25
Inflammation score	0.13 ± 0.10	1.0 ± 0.24[a]	0.75 ± 0.25[a]	1.33 ± 0.33[a]
EEL (mm^2)	6.44 ± 1.02	6.81 ± 0.76	8.15 ± 1.48[a,b]	6.73 ± 0.55
12 weeks	**n = 6**	**n = 6**	**n = 6**	**n = 6**
Neointimal thickness (mm)	0.15 ± 0.14	0.26 ± 0.26	0.25 ± 0.14	0.23 ± 0.12
Neointima area (mm^2)	1.50 ± 0.79	2.38 ± 1.99	2.27 ± 1.06	2.09 ± 0.66
% stenosis	28.7 ± 16.0	40.0 ± 23.0	36.1 ± 13.2	35.3 ± 11.5
Injury score	0.36 ± 0.26	1.20 ± 1.08	1.92 ± 1.30[a]	1.22 ± 0.21[b]
Inflammation score	0.33 ± 0.10	1.67 ± 0.57[a]	1.67 ± 0.33[a]	1.67 ± 0.33[a]
EEL (mm^2)	5.52 ± 0.76	6.94 ± 1.89	6.84 ± 0.89	6.88 ± 0.32

[a]$P \leq 0.02$ vs. bare stent.
[b]$P \leq 0.03$ vs. ceramic.

deployed at high pressure (10–14 atm for 20 sec) using a balloon 3.0–3.5 mm in diameter, 20 mm in length, for the 15 mm stent. The stent artery ratio was kept between 1:1.15 and 1:1.3. The position of the stent was documented angiographically. All animals were pretreated with aspirin 325 mg and clopidogrel 75 mg p.d. 24 hr before the procedure and on each subsequent day until death.

Stent Coating and Drug Loading Details

Stainless steel stents were coated with a 0.5 μm thick nanoporous aluminum oxide layer that houses and releases tacrolimus as described previously [16]. The maximal drug loading on such a nanoporous ceramic stent was 3 $\mu g/mm^2$ (Fig. 1A). Drug release kinetics show that about 80% of the initially loaded drug is released from the stent within 100 hr (Fig. 1B).

Tissue Processing and Histomorphometry

Animals were killed 1 and 3 months after stent placement. The hearts were excised and the treated arteries were perfusion-fixed with formalin. The stented segments were dissected free from the heart and embedded in methyl methacrylate. Specimens were sectioned with the stent in place with a rotational microtome (Leica, Germany). Vessel parameters were measured using a computerized image analysis program using 10× magnification as described previously [18,19]. In brief, area measurements were obtained by tracing the external elastic lamina (external elastic lamina area, EEL; mm^2), the internal elastic lamina (internal elastic lamina area, IEL; mm^2), and lumen line (LA; mm^2). Neointimal area (NA; mm^2) was calculated as follows: NA = IEL − LA. The percentage of neointimal stenosis was calculated as follows: % stenosis = IEL/LA × 100%.

Assessment of Vascular Injury and Inflammation

The extent of the vessel injury caused by the stents was quantified using the modified method of Schwartz et al. [20]. In brief, a score of 0–2 was applied to account for differences of mechanical injury in the media due to nonuniform strut expansion or nonproper stent coatings. The amount and extent of inflammatory cells around the stent struts was analyzed and scored as follows: no inflammation = 0; up to 1/4 of the neointima = 1; 1/4 to 1/2 of the neointima = 2; more than 1/2 of the neointima = 3.

Statistical Methods

Values are given in mean ± standard error (SEM). Comparisons of the three groups were made using either one-way ANOVA followed by the least-significant difference posthoc test for groups whose SD of the means was not statistically different ($P < 0.05$ by Bartlett's test) or by the Kruskall-Wallis. ANOVA followed by Dunn's test was used for groups whose SD of the means was statistically different ($P < 0.05$ by Bartlett's test). Statistical differences between treatment groups were considered significant with a $P < 0.05$.

RESULTS

Four weeks after implantation of either stainless steel (bare), ceramic aluminum oxide coating without drug release, 50 μg or 180 μg tacrolimus-eluting stents

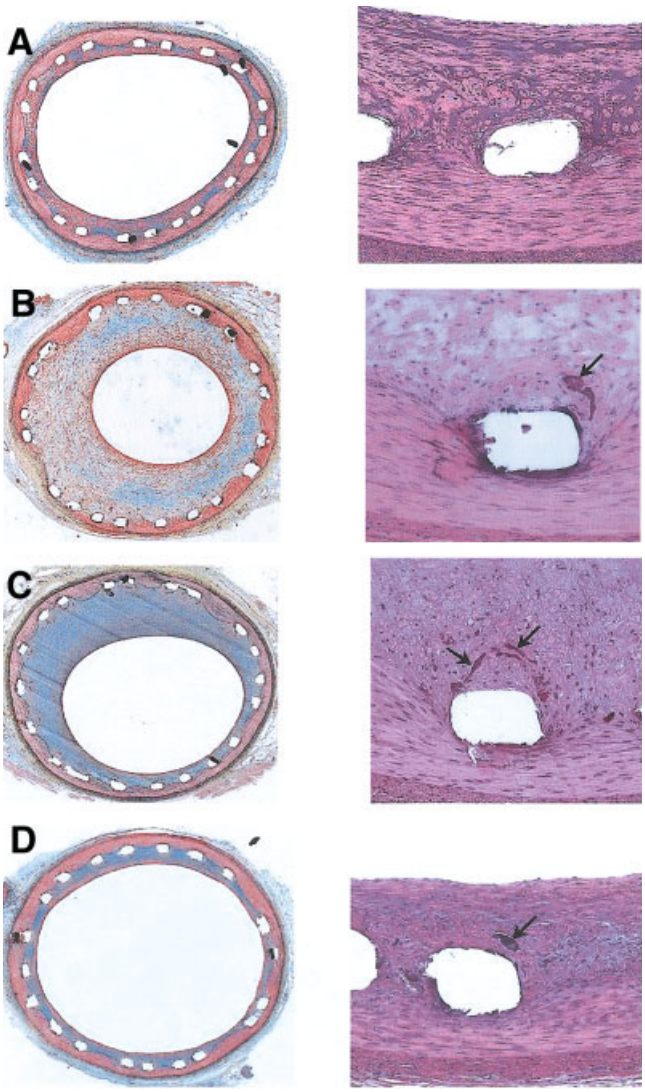

Fig. 2. Four histomorphometric images of arterial cross-sections processed 4 weeks after placement of bare stents, aluminum oxide ceramic-coated stents, and aluminum oxide ceramic-coated stent eluting 50 and 180 μg of tacrolimus (FK506). A: Bare metal stent at 4 weeks. Well-healed thin neointima present. B: Ceramic-coated stent at 4 weeks. Thick neointima present. Fragment of ceramic stent coating present in neointima (arrow, right). C: Ceramic-coated stent eluting FK506 (50 μg) harvested at 4 weeks. Well-healed moderately thick neointima present. Fragments of ceramic stent coating present in neointima (arrow, right). D: Ceramic-coated stent eluting FK506 (180 μg) harvested at 4 weeks. Well-healed neointima present; similar in area to bare metal stent. Fragment of ceramic stent coating present in neointima (arrow, right). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

(TESs), a significant increase of neointimal area was found with the nanoporous ceramic drug housing compared to the bare stents. Comparing both groups, neointimal thickness was two- to threefold higher and stenosis percent was twofold higher in the stent group with ceramic coating. Therefore, the combination of coating and drug failed to demonstrate efficacy in comparison with bare metal stents in a porcine model of restenosis. In all cross-sections of arteries containing stents coated with aluminum oxide layers, particle debris was noted in the media and neointima. The elution of 50 μg tacrolimus demonstrated a comparable increase in neointima area, intimal thickness, and stenosis percent as ceramic stent alone. Interestingly, the 180 μg dose TESs reduced neointimal growth significantly compared to stents with ceramic coating alone and eluting 50 μg indicating a potential antiproliferative effect of the drug. In the 4-week animals, injury score was comparable in all four groups examined. Inflammation scores were significantly higher after placement of stent with ceramic coating with and without elution of tacrolimus as compared to bare stents (Table I, Figs. 1–3).

Twelve weeks after placement of either stainless steel, ceramic, 50 μg and 180 μg TESs, we found similar results for intimal thickness, neointimal area, and stenosis percent compared with the 4-week groups. In all stents with ceramic coatings, an increased vascular inflammation was still present when compared with stainless steel stents (Table I, Figs. 1–3).

DISCUSSION

The present study demonstrated an inhibitory effect on neointimal proliferation of tacrolimus eluting from coronary stents in a porcine model of stent restenosis but revealed major particle debris from a nanoporous stent coating counteracting this inhibitory effect. Thus, we only confirmed in part previous animal data in the rabbit [16] showing that tacrolimus eluting from a nanoporous aluminum oxide stent coating may have antiproliferative capacities in the subgroup of the coated stents. The rabbit and the porcine model of restenosis have distinct differences with respect to vessel structure and proliferative responses to injury, and therefore the data of the present study are not comparable with the previous work. In our study, particle debris cracking off from the ceramic drug housing during stent expansion eliminated any potential antiproliferative effects of tacrolimus and, even worse, increased neointimal growth and luminal stenosis compared to bare stents. Our results may have been corroborated by the relatively high restenosis rates of a pilot clinical trial (PRESENT) using the nanoporous ceramic coating eluting tacrolimus (data not shown). The PRESENT trial was terminated early to refine the drug delivery platform of the stent. The aluminum oxide particle

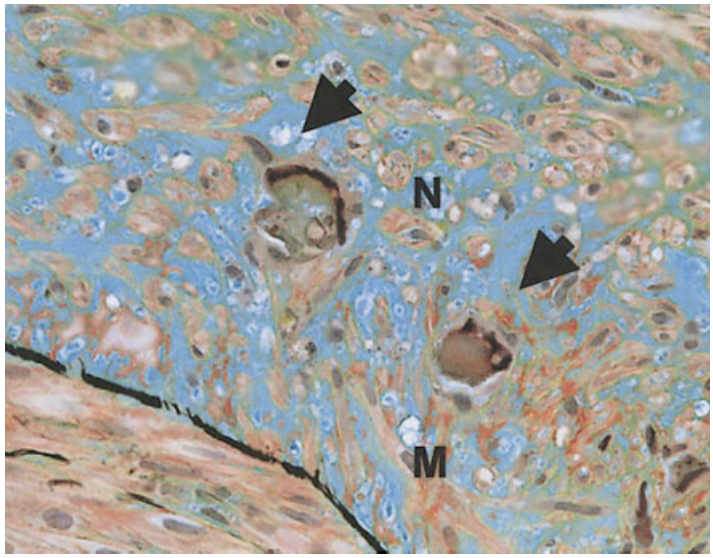

Fig. 3. High-power field magnification of the neointima 4 weeks after placement of a ceramic stent. The image shows aluminum oxide particles (arrows) within the neointima (N). M, media. [Color figure can be viewed in the online issue, which is available at www.interscience. wiley.com]

debris exaggerated inflammatory responses to the stent surface in porcine coronary arteries most likely during stent expansion. Mechanical stress during stent expansion and deployment in vivo may contribute to particle debris beyond the amount assessable by in vitro bench tests. An inflammatory response related to loose ceramic particles in other tissues was described by Nagase et al. [21].

Approximately 20% of the loaded tacrolimus (FK506) was not released from the stent, most likely because of the high liphophilicity of the drug. FK506 elicited a dose-dependent reduction of the neointima in the subgroup of stents with aluminum oxide coating, which appears to be associated with the known anti-inflammatory actions of the drug. Yet a relatively high dose of 180 μg dose of FK506 was necessary to yield amounts of neointima comparable with those formed in stainless steel stents. A neointimal catch-up was observed at 3 months in the 180 μg dose group vs. the results at 1-month follow-up, indicating that potential antiproliferative and anti-inflammatory drug effects may last only temporarily.

Other animal studies of drug-eluting stents have already shown that drug-eluting stents (DESs) may increase the presence of fibrin, inflammation, and incomplete endothelialization compared with bare stainless steel stents [22]. However, if inflammation is exaggerated in DESs, stent thrombosis, malapposition of the stent, as well as potential dislodgment may be the consequence. If drug-eluting stents are inflammatory, there may be greater risk of thrombus formation or hemorrhage around the strut. In the RAVEL study, for example, a higher incidence of incomplete stent apposition in patients receiving sirolimus-eluting stents compared with the uncoated stent group was found, which may not only be associated with the growth-arresting effects of the drug but also with a lack of biocompatibility of the polymer [12,23,24]. Studies looking at the time courses of inflammation after stenting suggested that lengthier human and animal studies are necessary to find out whether negative consequences will be observed in a late phase after implantation of drug-eluting stents. Animal studies of polymer-coated stents showed that healing is incomplete and inflammatory reactions are clearly still ongoing in a late phase after implantation of drug-eluting stents [25].

We previously reported that stent surface coatings may be prone to fissures, cracks, and particles debris [26]. The durability and biocompatibility of ceramic coatings may vary significantly by the substance and the manufacture of the coating. Titanium oxide, for instance, has other characteristics than aluminum oxide. Ceramic coatings of heart valves using titanium oxide have been shown to exert profound antithrombogenic and break resistant properties [27,28]. The present data suggest that stent coatings for drug release should be very tightly fixed to the stent frame. Many stent coatings, including those of polymer nature, come loose during the enormous mechanical stress of stent expansion and cause additional vessel wall injury. Local inflammation due to either particle elimination processes or augmented vascular injury is known to increase in stent restenosis. Further research is currently conducted to investigate whether surface porosity alone created in bare metal stent frames is eliminating the problem of particle debris cracking off from porous coatings of drug-eluting stents.

REFERENCES

1. Komatsu R, Ueda M, Naruko T, et al. Neointimal tissue response at sites of coronary stenting in humans: macroscopic, histological, and immunohistochemical analyses. Circulation 1998;98:224–233.
2. Kornowski R, Hong MK, Tio FO, et al. In-stent restenosis: contributions of inflammatory responses and arterial injury to neointimal hyperplasia. J Am Coll Cardiol 1998;31:224–230.
3. Waksman R, Robinson KA, Crocker IR, et al. Endovascular low-dose irradiation inhibits neointima formation after coronary artery balloon injury in swine: a possible role for radiation therapy in restenosis prevention. Circulation 1995;91:1533–1539.
4. Hehrlein C, Devries JJ, Arab A, et al. Failure of a novel balloon-expandable gamma-emitting (103)Pd) stent to prevent edge effects. Circulation 2001;104:2358–2362.
5. Drachman DE. Clinical experience with drug-eluting stents. Rev Cardiovasc Med 2002;3(Suppl 5):S31–S37.
6. Grube E, Gerckens U, Buellesfeld L. Drug-eluting stents: clinical experiences and perspectives. Minerva Cardioangiol 2002; 50:469–473.
7. Degertekin M, Serruys PW, Foley DP, et al. Persistent inhibition of neointimal hyperplasia after sirolimus-eluting stent implantation: long-term (up to 2 years) clinical, angiographic, and intravascular ultrasound follow-up. Circulation 2002; 106: 1610–1613.
8. Tanabe K, Serruys PW, Grube E, et al. TAXUS III trial: in-stent restenosis treated with stent-based delivery of paclitaxel incorporated in a slow-release polymer formulation. Circulation 2003;107:559–564.
9. Grube E, Silber S, Hauptmann KE, et al. TAXUS I: six- and twelve-month results from a randomized, double-blind trial on a slow-release paclitaxel-eluting stent for de novo coronary lesions. Circulation 2003;107:38–42.
10. Schwertz DW, Vaitkus P. Drug-eluting stents to prevent reblockage of coronary arteries. J Cardiovasc Nurs 2003;18:11–16.
11. Hong MK, Mintz GS, Lee CW, et al. Paclitaxel coating reduces in-stent intimal hyperplasia in human coronary arteries: a serial volumetric intravascular ultrasound analysis from the Asian Paclitaxel-Eluting Stent Clinical Trial (ASPECT). Circulation 2003;107:517–520.
12. Toutouzas K, Di Mario C, Falotico R, et al. Sirolimus-eluting stents: a review of experimental and clinical findings. Z Kardiol 2002;91(Suppl 3):49–57.
13. Sun J, Marx SO, Chen HJ, et al. Role for p27(Kip1) in vascular smooth muscle cell migration. Circulation 2001;103:2967–2972.
14. Thomson AW, Bonham CA, Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. Ther Drug Monit 1995;17:584–591.
15. Murphy JG, Schwartz RS, Edwards WD, et al. Percutaneous polymeric stents in porcine coronary arteries: initial experience with polyethylene terphalate stents. Circulation 1992;86:1596–1604.
16. Wieneke H, Dirsch O, Sawitowski T, et al. Synergistic effects of a novel nanoporous stent coating and tacrolimus on intima proliferation in rabbits. Catheter Cardiovasc Interv 2003; 60: 399–407.
17. Schwartz RS, Edelman ER, Carter A, et al. Drug-eluting stents in preclinical studies: recommended evaluation from a consensus group. Circulation 2002;106:1867–1873.
18. Farb A, John M, Acampado E, et al. Oral everolimus inhibits in-stent neointimal growth. Circulation 2002;106:2379–2384.
19. Farb A, Heller PF, Shroff S, et al. Pathological analysis of local delivery of paclitaxel via a polymer-coated stent. Circulation 2001;104:473–479.
20. Schwartz RS, Huber KC, Murphy JG, et al. Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. J Am Coll Cardiol 1992;19:267–274.
21. Nagase M, Baker DG, Schumacher HR Jr. Prolonged inflammatory reactions induced by artificial ceramics in the rat air pouch model. J Rheumatol 1988;15:1334–1338.
22. Farb A, Heller PF, Shroff S, et al. Pathological analysis of local delivery of paclitaxel via a polymer-coated stent. Pathological analysis of local delivery of paclitaxel via a polymer-coated stent. Circulation 2001;104:473–479.
23. Sharma S, Bhambi B, Nyitray W. Sirolimus-eluting coronary stents. N Engl J Med 2002;347:1285.
24. Sousa JE, Costa MA, Abizaid A, et al. Lack of neointimal proliferation after implantation of sirolimus-coated stents in human coronary arteries: a quantitative coronary angiography and three-dimensional intravascular ultrasound study. Circulation 2001;103:192–195.
25. Virmani R, Liistro F, Stankovic G, et al. Mechanism of late in-stent restenosis after implantation of a paclitaxel derivate-eluting polymer stent system in humans. Circulation 2002; 106: 2649–2651.
26. Hehrlein C, Zimmermann M, Metz J, et al. Influence of surface texture and charge on the biocompatibility of endovascular stents. Coron Art Dis 1995;6:581–586.
27. Changrong L, Zhihong Z, Fuming Z, et al. TiO2 films prepared by ion beam assisted deposition. Nucl Instr Med Phys Res 2000;169:21–25.
28. Wang X, Zhang F, Changrong L, et al. Improvement of blood compatibility of artificial heart valves via titanium oxide film coated on low temperature isotropic carbon. Surface Coatings Technol 2000;128:36–42.