Original article

Investigation of long-term implantation of BuMA stent in a porcine coronary model

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Background Stent-based delivery of sirolimus has been shown to reduce neointimal hyperplasia significantly. However, the long-term effect of the polymer is thought to initiate and sustain an inflammatory response and contribute to the occurrence of late complications. Our study aimed to evaluate the efficacy and safety of the BuMA biodegradable drug-coated sirolimus-eluting stent (BSES) for inhibiting neointimal hyperplasia in a porcine coronary model.

Methods Four types of stents were implanted at random in different coronary arteries of the same pig: BSES (*n*=24), bare metal stent (BMS) (*n*=24), biodegradable polymer coated stent without drug (PCS) (*n*=24) and only poly (n-butyl methacrylate) base layer coated stent (EGS) (*n*=23). In total, 26 animals underwent successful random placement of 95 oversized stents in the coronary arteries. Coronary angiography was performed after 28 days, 90 days and 240 days of stent implantation. After 14 days, 28 days, 90 days and 240 days, 6 animals at each timepoint were sacrificed for histomorphologic analysis.

Results The 28-day, 90-day and 240-day results of quantitative coronary angiography (QCA) showed reduction in luminal loss (LL) in the BSES group when compared with the BMS group; (0.20±0.35) mm vs. (0.82±0.51) mm (*P*=0.035), (0.20±0.30) mm vs. (0.93±0.51) mm (*P*=0.013), and (0.18±0.16) mm vs. (0.19±0.24) mm (*P*=0.889), respectively. By 28-day, 90-day and 240-day histomorphomeric analysis results, there was also a corresponding significant reduction in neointimal tissue proliferation with similar injury scores of BSES compared with the BMS control; average neointimal area (0.90±0.49) mm² vs. (2.16±1.29) mm² (*P*=0.049), (1.53±0.84) mm² vs. (3.41±1.55) mm² (*P*=0.026), and (2.43±0.95) mm² vs. (3.12±1.16) mm² (*P*=0.228), respectively. High magnification histomorphologic examination revealed similar inflammation scores and endothelialization scores in both the BSES and BMS groups.

Conclusions The BuMA biodegradable drug-coated sirolimus-eluting stents can significantly reduce neointimal hyperplasia and in-stent restenosis. Re-endothelialization of the BuMA stent is as good as that of the BMS in the porcine coronary model due to the reduced inflammation response to the BuMA stent.

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The combination of the drug with the stent is the most effective means of prevention and intervention treatment of restenosis, and has been shown to significantly reduce the incidence of restenosis in routine clinical practice. Ninety percent of the drug-eluting stents use polymers as the carrier of the drug, and the polymeric coating can also control the drug elution kinetics to achieve an optimal drug release profile *in vivo*. From the current short-term statistical data, the drug-eluting stents (DES) have significantly reduced the incidence of restenosis compared with bare metal stents from 30% to less than 10%.[1-4] But with the increasing number of patients receiving the intervention treatment, the long-term safety of DES is more of a concern after implantation, especially the impact of non-degradable polymers on the vascular response.[5] Drug residues, the polymer induced hypersensitivity reactions, and the coating peeling or cracking during stent implantation cause concerns that late thrombosis is a result of the unhealed endothelium. Biodegradable polymeric drug coated stents should be able to solve the problems of side-effects from drug residues and the durable polymer, and also an advanced coating technology is needed to improve the coatings' mechanical integrity to avoid the coating cracking and delaminating during the operation. This will be the main trend in DES development in the next few years.

The BuMA biodegradable drug coating coronary stent system contains two different functional coating layers: the electro-grafting base layer (poly (n-butyl methacrylate) coating) and the biodegradable poly (lactide-co-glycolic acid) (PLGA) drug carrier. The base layer is electro-grafted onto the bare metal stent surface with a thickness around 100 to 200 nm, and has strong adhesion and excellent uniformity on the complex stent surface. The base layer secures adhesion of the biodegradable PLGA matrix, and prevents the coating

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from cracking and delamination upon its expansion as a result of the interdigitaion between the surface-anchored base layer polymer chains and the top PLGA matrix. The base layer also has the benefit of suppressing corrosion and ion release from the metal stent substrates which could contribute to a lower local inflammation response *in vivo*. With the supporting base layer and the gradual degradation of the PLGA polymer, the BuMA stent has achieved an excellent coating mechanical integrity and 100% release of the drug substance, thus it should reduce the late stent thrombosis, in stent restenosis and other problems caused by the unhealed endothelium and local inflammation response. This study was to evaluate the efficacy and safety of this novel stent in a porcine coronary model.

METHODS

Stents preparation

The bare metal stent (BMS) was made of 316L stainless steel (provided by SINO Medical Sciences Technology Inc.). The stent was laser cut from seamless tubing and electropolished.

The base layer coated stent (EGS) was based on the BMS platform and featured an electro-grafted poly(n-butyl methacrylate) polymer with a thickness of 100 to 200 nm.

The biodegradable polymer coated stent (PCS) was based on the EGS and featured a sprayed PLGA drug carrier without drug substance. The degradation period of the PLGA polymer was around 10 weeks *in vivo*.

The BuMA biodegradable drug coating sirolimus-eluting stent (BSES) was based on the EGS and featured a sprayed PLGA drug carrier with sirolimus. Sirolimus was completely released in a controlled manner for 30 days *in vivo*.

Animal preparation and stents implantation

Twenty-six female Chinese miniature pigs (6 months to 8 months old, 25 to 30 kg) were provided by Beijing Agricultural University. Three days before implantation, aspirin (Bayer, Germany) 300 mg and clopidogrel (Xinli Tai Pharmaceutical Co. Ltd, Shenzhen, China) 75 mg were administered daily. Preoperative Ketamine (0.3 mg/kg) by a subcutaneous injection along with 0.05 g/L sodium pentobarbital by intravenous injection was used for basic anesthesia. A 6F sheath was inserted through the femoral artery. Heparin (200 U/kg) was administered intra-arterially via the sheath. A 6F JL 3.5 guiding catheter was used for coronary angiography. The stent size was selected according to the target vessel diameter (stent diameter: vascular diameter, 1.1:1.0–1.2:1.0). A 0.014 inches guide wire was sent to the distal coronary artery. The stent was placed in the target vessel segment and inflated at 8–12 atm for 10 to 20 seconds. The angiography procedure was repeated and no serious tear or thrombosis was observed. The sheath was withdrawn slowly and we compressed the puncture site for 20–30 minutes to stop bleeding. Intramuscular injection of penicillin (8×10^5 U/kg) was given. After the procedure, all the animals were treated with aspirin (300 mg) and clopidogrel (75 mg) daily until sacrifice.

Coronary angiography and quantitative analysis of coronary artery stenosis

At 28, 90 and 240 days after implantation six animals were evaluated. Coronary analysis was conducted by quantitative image analysis software (GE Medical Devices Co., Ltd. Germany). Coronary artery measurements were recorded, including baseline vessel diameter and minimal lumen diameter immediately after implantation, reference vessel diameter, and minimal lumen diameter were recorded at each time point to calculate the lumen loss.

Pathological analysis

At 14, 28, 90, and 240 days after implantation, six animals were sacrificed. The heart was collected and heparin saline perfusion was conducted for 5 minutes with a perfusion pressure of 100 mmHg (1 mmHg=0.133 kPa). We quickly separated the stent vessel segment and fixed it with 10% formaldehyde solution. The segments were embedded in methy methacrylate plastics for histological examination.

Slicing and staining

The samples with stents were embedded in methyl methacrylate (prepared by Peking University Dental Hospital), and then sliced at 100 μm with a hard tissue microtome (LEICA SP1600 hard tissue slicer, Germany). All sections were stained with H&E for light microscopy examination.

Image analysis

LEICA DFC 300FX optical microscope and LEICA Qwin PLUS V3.2.1 image analysis software were used to measure the lumen area, internal elastic lamina area, external elastic panel area, neointimal area (internal elastic lamina area–lumen area), and the percentage area of restenosis ((internal elastic lamina area–lumen area)/lumen area × 100%). Vascular injury score was determined via the Schwartz method: 0=intact internal elastic lamina, 1=internal elastic lamina fracture, 2=internal elastic lamina and tunica media fracture, 3=external elastic plate fracture.[6] Inflammation score was graded by the inflammatory cells; 0=no inflammatory cells, 1=scattered inflammatory cells, 2=inflammatory cells encompassing 50% of a strut in at least 25%–50% of the circumference of the artery, 3=inflammatory cells surrounding a strut in at least 25%–50% of the circumference of the artery.[7] The stent endothelialization score was defined as the extent of the circumference of the arterial lumen covered by endothelial cells and graded from 1 to 3: 1=25%, 2=25%–75%, 3≥75%.[8]

Statistical analysis

Values were expressed as mean±standard deviation (SD). Group imaging, histological and integral data were tested

with the analysis of variance (ANOVA) test. SPSS10.0 statistical software (SPSS Inc., USA) was used for analysis. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

General data

A total of 103 stents including four different types were implanted in a total of 26 pigs in this study. Two pigs died due to respiratory depression in surgery; the anatomy showed no stent thrombosis. A total of 95 stents in 24 pigs remained (Table 1) after the procedure. Fourteen days after stent implantation, six animals were sacrificed for the histomorphometry examination and the evaluation of endothelialization. At 28 days, 90 days and 240 days after stent implantation, six animals received coronary angiography and were then sacrificed for the histomorphometry examination and the evaluation of endothelialization.

Quantitative image analysis of coronary angiography

The reference vessel diameter (RVD) for all groups was similar at baseline. The lumen loss in the BSES group was significantly lower than that in the BMS group at day 28 and day 90 when determined by QCA after stent implantation (Table 2). But the QCA results at day 240 showed no significant differences among groups.

Results of pathological analysis

LEICA Qwin PLUS V3.2.1 image analysis software was used for quantitative analysis of the lumen area, internal elastic lamina area, external elastic panel area and neointimal area (internal elastic lamina area–lumen area). The neointimal area in the BSES group was significantly less than in the BMS group by image analysis at day 28 and day 90 (Table 3). The neointimal area in the BSES group was lower than that in the BMS group at day 240.

Results of injury score, inflammation score and stent endothelialization score were similar among all groups at day 14, day 28, day 90 and day 240 after implantation (P >0.05, Table 4).

Thrombosis observance

There were no clear signs of thrombosis in any group at day 14, day 28, day 90 and day 240 after stent implantation.

Table 1. Status of the stent implantation

Types	Number	LAD	LCX	pRCA	dRCA
BMS	26	8	5	8	5
PCS	26	5	9	5	7
BSES	26	6	7	5	8
EGS	25*	7	5	7	6

*Due to the abnormal blood vessel of one experimental animal, there was lack of one EGS stent in this study.

Table 2. The QCA results of day 28, 90 and 240 after stent implantation

Types	Reference vessel diameter (mm)	MLD immediately after implantation (mm)	MLD by QCA (mm)	Lumen loss (mm)
Day 28				
BMS (*n*=6)	2.80±0.71	2.70±0.17	1.88±0.59	0.82±0.51
BSES (*n*=6)	2.88±0.30	2.70±0.28	2.50±0.48	0.20±0.35*
EGS (*n*=6)	2.83±0.28	2.62±0.20	2.17±0.39	0.45±0.26
PCS (*n*=6)	2.84±0.22	2.78±0.37	1.98±0.47	0.80±0.41
Day 90				
BMS (*n*=6)	2.81±0.37	2.63±0.15	1.70±0.44	0.93±0.51
BSES (*n*=6)	3.05±0.37	2.78±0.31	2.58±0.15	0.20±0.30†
EGS (*n*=6)	2.95±0.32	2.80±0.32	2.52±0.33	0.28±0.34
PCS (*n*=6)	2.89±0.38	2.80±0.32	1.25±0.80	1.47±0.75
Day 240				
BMS (*n*=6)	2.69±0.20	2.91±0.10	2.72±0.17	0.19±0.24
BSES (*n*=6)	2.61±0.17	2.73±0.29	2.55±0.33	0.18±0.16‡
EGS (*n*=5)	2.77±0.20	3.17±0.37	3.04±0.31	0.13±0.08
PCS (*n*=6)	2.59±0.29	2.83±0.26	2.57±0.39	0.27±0.21

*P=0.035, †P=0.013, ‡P=0.889, BSES group vs. BMS group.

Table 3. Pathological analysis results

Types	Lumen area (mm^2)	Area around the internal elastic laminae (mm^2)	Neointimal area (mm^2)
Day 28			
BMS (*n*=6)	4.71±1.24	6.88±1.55	2.16±1.29
BSES (*n*=6)	4.93±0.95	5.84±1.07	0.90±0.49*
EGS (*n*=6)	3.65±1.55	5.24±0.91	1.59±1.01
PCS (*n*=6)	2.65±1.54	5.69±0.69	3.04±1.97
Day 90			
BMS (*n*=6)	2.68±2.06	6.10±1.31	3.41±1.55
BSES (*n*=6)	3.70±1.76	6.09±1.39	1.53±0.84†
EGS (*n*=6)	3.66±1.08	6.38±1.25	2.72±1.71
PCS (*n*=6)	2.60±2.70	6.32±1.48	3.71±2.81
Day 240			
BMS (*n*=6)	1.89±0.66	5.02±0.89	3.12±1.16
BSES (*n*=6)	2.62±0.80	5.05±0.55	2.43±0.95‡
EGS (*n*=5)	4.19±0.54	6.12±0.24	1.92±0.44
PCS (*n*=6)	1.22±0.76	5.01±0.95	3.79±0.92

*P=0.049, †P=0.026, ‡P=0.288, BSES group vs. BMS group.

Table 4. Injury score, inflammation score and stent endothelialization score

Types	Inflammation score	Injury score	Endothelialization score
Day 14			
BMS (*n*=6)	1.33±0.52	1.83±0.75	3.00±0.00
BSES (*n*=6)	1.50±0.55	1.17±0.41	2.61±0.49
EGS (*n*=6)	1.17±0.41	1.33±0.52	2.78±0.40
PCS (*n*=6)	1.83±0.75	1.66±0.52	2.72±0.44
Day 28			
BMS (*n*=6)	0.95±0.13	1.50±0.55	3.00±0.00
BSES (*n*=6)	1.33±0.52	1.67±0.52	2.67±0.56
EGS (*n*=6)	1.17±0.41	1.17±0.41	2.50±0.55
PCS (*n*=6)	1.19±0.52	1.33±0.82	2.59±0.48
Day 90			
BMS (*n*=6)	1.17±0.41	1.96±0.64	3.00±0.00
BSES (*n*=6)	2.17±0.75	2.17±0.75	3.00±0.00
EGS (*n*=6)	1.50±0.84	1.58±0.49	3.00±0.00
PCS (*n*=6)	1.83±0.75	1.83±0.76	3.00±0.00
Day 240			
BMS (*n*=6)	2.50±0.55	2.50±0.84	3.00±0.00
BSES (*n*=6)	2.00±0.89	2.00±0.63	3.00±0.00
EGS (*n*=5)	2.40±0.55	2.60±0.55	3.00±0.00
PCS (*n*=6)	2.67±0.52	2.67±0.52	3.00±0.00

DISCUSSION

Large scale clinical trials have confirmed that sirolimus-eluting stents can significantly reduce the

in-stent restenosis rate.[1-3] However, some studies showed that the first generation drug-eluting stents can in the long term lead to late stent thrombosis caused by an inflammatory response induced by non-degradable polymer stimulation and the unhealed endothelium.[9]

The biodegradable polymer drug-eluting stent may avoid the inflammatory response described above. The polymer coating degrades along with the release of the drug. In some animal studies, the biodegradable polymer drug-eluting stent has shown promising results.[10] In this study, coronary angiography and histological analysis showed that, at day 28, day 90 and day 240 after stent implantation, the lumen loss and the neointimal area were significantly less in the BSES group than that in the BMS group, which suggested that the BuMA stent can effectively reduce the neointimal hyperplasia and the stent restenosis *in vivo*.

Restenosis can be considered a vessel wall inflammatory repair response after injury. Accompanying inflammatory cells aggregate around the stent adhesion, platelets are activated and release multiple cytokines and growth factors that lead to smooth muscle cells migration and proliferation.[11] Therefore, the control of the inflammation response theoretically can reduce the in-stent restenosis after stent implantation. Currently the studies of blood vessel inflammation after stent implantation are mostly done in the miniature porcine model. Some studies suggest that the porcine and human vascular repair response after injury is different. After stent implantation, the peak of neointimal hyperplasia in porcine coronary arteries is within 1 month, but in humans it is around 3 to 6 months. After stent implantation, the endothelialization of the porcine coronary artery is completed in 90 to 240 days, which is equal to 2 or 3 years in the human coronary artery after PCI.[11] We are more concerned about late stent thrombosis and in-stent restenosis after DES implantation, therefore, the inflammatory response and endothelialization evaluation may need to be considered at day 90 and day 240 in porcine coronary model. Carter found that in the miniature porcine coronary model, compared to the bare metal stents, sirolimus DES significantly reduced neointimal proliferation in 30 days, but at 90 days and at long term follow-up, the results between the DES and BMS groups were similar. At the same time, 90 days and long term results of the inflammation scores for the DES were significantly higher than for the bare metal stents. Therefore, Carter et al considered that the late inflammatory response induced delayed cell proliferation, which counteracted the inhibition effect of the sirolimus.[12] In some domestic biodegradable DES animal studies, at 90 days and longer follow-up, inflammation scores in the DES group were often higher than in the BMS group, and neointimal proliferation was similar between the two groups. That may be explained by the polymer or bare metal stimulation induced inflammatory response and unhealed endothelium.

In this study, the analysis of the inflammation score in the BSES group at 240 days was slightly lower than in the BMS group, and this is different from previous studies.[12] The possible reason is that after the degradation of the biodegradable coating, the poly (n-butyl methacrylate) base layer can reduce the degree of the inflammatory response induced by the polymer or bare metal stents. In this study, the results in the PCS and EGS groups showed us evidence of this. Although the lumen loss and the inhibition of neointimal proliferation in the two groups showed no significant advantage over that in the BMS group in the early stage, the inflammation score in the EGS group showed a descending trend over the BMS group. At 240 days, the inflammation score in the EGS group was lower than in the BMS group.

Virmani et al[5-13] considered that the late stent thrombosis was closely related to delayed or unhealed endothelialization. In this study, the endothelialization score in the BSES group was slightly lower than that of the BMS group at 14 days and 28 days, but no significant differences were observed. At 90 days and 240 days, all of the stents were fully endothelialized without observed necrosis, vascular tumors or in-stent thrombosis. Therefore, the BuMA biodegradable drug coating stent has shown excellent biocompatibility in terms of the degree of inflammatory response and re-endothelialization after stent implantation. In addition, according to the results of animal study conducted by Dr. Virmani (data provided by SINO Medical Sciences Technology Inc.), the BSES stents and EGS stents also showed excellent re-endothelialization results in the rabbit iliofemoral model. Nearly complete re-endothelialization was achieved for the BMS and the EGS stents 14 days after the implantation. And also nearly complete re-endothelialization was achieved for the BSES stents 90 days after the implantation. The results from the rabbit model were consistent with ours.

The artery of porcine coronary model did not have coronary atherosclerosis, therefore we cannot accurately simulate human coronary atherosclerotic lesions in this study. The sample size may affect the results of statistical analysis and there is no further observation in a longer-period in this study. There may be more obvious trends we would find in a longer study.

In summary, the animal experiments suggest the BuMA biodegradable drug coating stent can safely and effectively reduce the neointimal hyperplasia and in stent restenosis. Re-endothelialization of the BuMA stent is as good as that of the BMS in the porcine coronary model due to the reduced inflammation response to the BuMA stent.

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