Ultra-microstructural changes in iliac artery after bare and magnetic stent implantation in rabbits

Xinhong Guo¹, Guoliang Jia², Anlin Lu², Xinguo Zhao², Fei Li², Rongqing Zhang²

1 Institute of Geriatric Cardiology, Chinese PLA General Hospital, Beijing 100853, China
2 Department of Cardiology, Xijing Hospital, Xi’an 710033, China

Objective To investigate the preventive effect of magnetic stent on coronary restenosis after percutaneous arterial stenting.

Methods Twenty rabbits were divided randomly into 2 groups. Bare stent (BS group, n=10) or magnetic stent (MS group, n=10) was implanted in the left iliac artery of the rabbits of the 2 groups, respectively. Aspirin (25mg, qd) was administered orally to the rabbits of both groups from 3 days before stenting until the rabbits were executed. Unfractionated heparin (2500u, qd) was delivered subcutaneously after stenting for 7 days. Five rabbits of each group were randomly selected to be executed at 7 or 30 days. Structural changes in the injured arteries were studied by optical microscopy, transmissive electronic microscopy and immunohistochemistry.

Results At 7 days, more myofibroblasts were found migrating from adventitia to tunica media and intima in BS group than in MS group. Inside the media and intima, large amount of smooth muscle cells of synthetic type were observed. At 30 days after stenting, in magnetic group, most vascular smooth muscle cells (SMCs) under the intima had transformed to contractile type and only little extracellular matrix (ECM) was observed around the SMCs; whereas, in BS group, the SMCs remained to be synthetic type and large amount of ECM was observed around the SMCs, which was composed mainly of proteoglycans and glycoproteins.

Conclusions Magnetic stent can inhibit proliferation and migration of SMCs and reducing the production of ECM, and therefore, may prevent restenosis after coronary stenting. (J Geriatr Cardiol 2008; 5:182-185)

Key words coronary atherosclorosis disease; stent restenosis; extracellular matrix; smooth muscle cell

Introduction

The use of stents since the 1990’s has improved the results of percutaneous coronary revascularization.¹,² However, in-stent restenosis continues to limit the long-term success of this approach.³,⁴ Drug-eluting stents, which were introduced several years ago, reduce the need for subsequent revascularization procedures as compared with bare-metal stents, by reducing neointimal hyperplasia after vascular injury, hence decrease late luminal loss and angiographic restenosis.⁵,⁶ Despite these benefits, drug-eluting stents may engender adverse arterial responses, including delayed endothelialization and hypersensitivity to the polymeric coating that regulates drug dose and release kinetics. Recent reports from randomized trials and observational studies using historical controls have suggested that drug-eluting stents be associated with increased rates of late stent thrombosis and death, as compared with bare-metal stents.⁷ Therefore, exploring more biologically friendly, restenosis-reducing stents is warranted.

Magnetic fields have broad biological effects, including modulating calcium concentration inside the myocardial cells,⁸ protecting myocardial cells,⁹ regulating transcription and translation of several proteins in cultured cell, improving endothelial cells function,¹⁰ and inhibiting vascular smooth muscle cells (SMCs) proliferation.¹¹ Many enzymes, exposed under the magnetic field, have the tendency of enhanced activity.⁹ Thus, we developed a new type of intra-coronary stent-magnetic stent. Our previous animal research and phase I clinic study have shown decreased in-stent restenosis and other clinical side-effects after implantation of magnetic stent as compared with bare stent.¹² However, the mechanisms underlying these beneficial effects have not been investigated. We hypothesized that the mechanism for magnetic stent to prevent in-stent restenosis may relate to inhibition of proliferation and migration of SMCs and reducing the production of ECM. In this study, we aimed to examine the ultra-microstructural changes in the injured arteries after magnetic and bare stent implantation.

Materials and methods

Magnetic stent

NIR intra-coronary stent(Bosten, USA) was used for magnetic stent in this study. Bare stent is demagnetized by the manufacturer before it is sold, therefore it doesn’t have magnetism. However, it can be magnetized under some special condition, and be made to magnetic stent. Stent magne-
tization and test technique of magnetic stent have been patented in China (patent number: 02262335). The average magnetic field intensity is 30-50 milli-Gauss after the stent being magnetized. The magnetism usually lasts for 12-24 months.

Study protocol

Study animals were purchased from Experimental Animal Center of the Forth Military Medical University. All experiments were performed in accordance with the guidelines for animal research. Twenty New Zealand white rabbits of both sexes, 4-12 month aged, weighing 2.5-3kg, were randomly divided into magnetic stent (MS,n=10) and bare stent (BS,n=10) groups. Anesthesia was performed with sodium pentobarbital (30mg/kg). The protocol for angioplasty and stent implantation was described previously. In brief, right jugular artery was exposed, a 5F arterial catheter was inserted into the right jugular artery. Heparin (2500u) was given intra-arterially. A 0.035inch J form guidewire was advanced to abdominal aorta by the artery sheath. Gently advanced a 5F Judkins right coronary angioplasty catheter along the guidewire, selective abdominal aorta cineangiogram was taken and then took out the angioplasty catheter. A 0.014inch guidewire was advanced to the left iliac artery through the sheath. A NIR stent was cut into two pieces at the bridging strut, and a single piece (10-mm length) was mounted on a PTCA balloon, ratio of artery and balloon was 1.0:1.2, advanced the stent mounted balloon to left iliac artery that parallel to the first lumbar, inflated for 30 seconds, at 8-10 atm, withdrew the balloon, repeated angiography. The rabbits received a dose of 25mg aspirin orally per day for 3 days before operation and till they were executed. After the operation, heparin, at a dose of 2500u, was given subcutaneously for 1 week.

Tissue collection and fixation

Five rabbits from each group were selected randomly to be executed by a lethal dose(120mg/kg) of sodium pentobarbital at 7 and 30 days after stenting, respectively. The abdominal aorta was cannulated and the animals were exsanguinated by flushing with physiological saline solution at 100 mmHg pressure. The left iliac arteries were harvested, cleaned of any adipose tissue, and divided into 2 segments. One is fixed in 4% paraformaldehyde, embedded in paraffin and used for optical microscopic examination; the other segment was fixed with 3% glutaraldehyde solution and used for transmissive electronic microscopic examination.

Optical microscopic examination

Morphometric analysis was performed on 3 hematoxylin-eosin-stained cross sections for each stented segment of artery. Intimal thickening was quantified by computer-based icon analysis system. The level of vessel injury was evaluated by the standard report by Schwarts et al.[9] Transmissive electronic microscopy observation

After fixed with 3% glutaraldehyde solution, the arterial segments were then fixed in 1% osmium tetroxide and 0.1 mol/L cacodylic acid, for 1 to 2 hours, dehydrated in graded ethanol baths, and embedded in epoxy-araldite. Specimens were sectioned into 500-µm slices and stent struts were removed. The segments were then re-embedded in epoxy-araldite, and four segments of each group of the stented arteries were cut into 40-to 80-nm sections. The sections were mounted on copper nets and 2% uranyl acetate and lead citrate added for contrast. Then the specimens were examined on transmissive electronic microscopic (JEM-2000EX, Japan).

Statistical Analysis

Data are expressed as mean±SD. A value of P<0.05 was considered significant.

Results

All vessels under stent implantation showed internal elastic lamina rupture, media thinner and laceration. Average scores of vessel injury were 2.03±0.11 and 2.12±0.14 in BS and MS, respectively. There is no significant difference among the two groups(P>0.05).

Under optic microscopy, inner lumen of BS group’s iliac arteries was rough, intima hyperplasia was significant, and mainly composed of VSMCs and there is a lot of ECM formation. Endothelial cell arrangement was irregular and the internal elastic laminae were un-intact. There is no significant difference between media and adventitia. In MS group, the inner lumen was smoother than that of BS group, with only a little bit intima hyperplasia.

At 7 days after stenting, there were more myofibroblasts migrated from adventitia to media and intima in BS group than those in MS groups. Inside the media and intima, there were many synthetic type SMCs (Figure 1), ECM composed mainly collagen and elastin. There are few lysosomes in the foam cells under the intima, myofilaments and basement membrane could be seen inside the foam cell, which suggests that the foam cells be originated from SMC(Figure 2); many myofibroblasts can be seen in both media and adventitia. Inside the myofibroblasts, rough endoplasmic reticulum and myofilaments can be seen, but there is no dense patch and dense body (Figure 3).Whereas the neointima in MS group mainly infiltrated with inflammation cells such as macrophage cells, the shape of cells is irregular, lysosomes and vacuole structure can be seen, with small nucleolus. A few degenerated SMCs transformed to contractile type, with dilated endoplasmic reticulum and obviously swollen mitochondria, and some extracellular membrane structures can be seen around the cells, with small amount of collagen and elastin (Figure 4). There are more lysosomes in the foam
cells under the intima, myofilaments and basement membrane could not be seen inside the foam cell, which suggests that the foam cells be originated from macrophages (Figure 5); a 30 days, SMCs under the intimae in BS group were still synthetic type, with abundant ECM around them, which was mainly composed of proteoglycans and glycoproteins. There are lots of synthetic type SMCs in media and abundant rough endoplasmic reticulum inside the cells. Myofibroblasts migrating from adventitia to media or to intima could be observed. In contrast, SMCs under the intima or in media in MS group were of degenerated contractile type and inactive macrophages, there was little collagen and elastin around the cells (Figure 6). Few myofibroblasts can be found.

**Discussion**

In the present study, we found that proliferation ex-
tent of SMCs in neointima in the iliac artery of the rabbits in MS group is much lower than that of BS group. It seems that MS can prevent in-stent restenosis by inhibiting proliferation of SMC. We found that there were more macrophages in intimae and media of artery in MS group, less SMCs and more SMCs appeared as contractile phenotype, while there were fewer macrophages, a large amount of proliferated synthetic type SMCs, and a lots of glycoprotein and proteoglycans in intimae and media of artery in BS group. These observations suggest that MS prevent in-stent restenosis by controlling the transforming of SMCs to synthetic type, hence inhibiting proliferation of SMCs. We also found that ECM in neointima of BS group was mainly composed of glycoprotein, which further enhanced proliferation, migration, phenotype transforming and ECM secretion ability of SMCs. While in MS group, ECM was mainly composed of collagen and elastin, which could restrain migration of SMC. Therefore, we suspected that different components of ECM may be the reason for lower proliferation of SMC and ECM fewer number of SMCs in neointima of MS group. Previous studies of in-stent restenosis had focused on media and intima. More recently, however, the effect of adventitia on restenosis has been paid more attention. By observing the migration of myofibroblast in the outer layer of artery vessel, we found that there were more myofibroblasts which transformed by fibroblasts migrated from media to intimae in BS group than that in MS group. If myofibroblasts in neointima come from adventitia, MS may prevent in-stent restenosis by inhibiting migration of myofibroblasts in adventitia to neointima. The mechanism needs further investigation.

In conclusion, based on the observation of vascular ultra-structure after MS and BS implantation, we found that the preventive effect of magnetic stent on coronary restenosis may be explained, at least in part, by the effects of MS on inhibiting the transformation of SMC to synthetic type, decreasing early formation of ECM, and inhibiting migration of myofibroblasts to the intima.

Reference