The Studies on Biocompatibility of Self-expanding NiTi Stent and Apoptosis of Smooth Muscle Cells after Stenting

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Abstract. The biocompatibility of the NiTi alloy self-expanding stent, its dilating effect on the vascular wall, and the apoptosis of smooth muscle cells (SMCs) were studied by implantation of stent into the rabbit’s abdominal aorta for different period. All the animals lived throughout the study. There was no detectable migration or dissection of the stent, and there were no acute closures or sub-acute thromboses in the vessels. The rates of patency were 100% both at the beginning when the stent was implanted and at the end when the animal was sacrificed. It may be concluded that the vascular intima covers the whole stent at the 8-week point. The atherosclerotic process existed in the vascular intima in contact with the stent surface, while the proliferation and apoptosis of SMCs occurred simultaneously. After stent implantation, the apoptosis happened in both intima and media, which indicated that the stent might not only stimulate the intima but also compress the media, leading to proliferation and apoptosis. This might contribute to vessel remodeling after stenting.

Introduction

NiTi stent could be made into shape memory type, superelastic type or retrieved type. The transition temperature of the shape memory type stents in previous studies[1,2] were between 45°C~50°C, which allowed the stent to maintain in a proper contraction state during the delivery without protective sheath, and the expansion of the stent was realized by injecting a high temperature saline water. In the present study, superelastic self-expanding stent were utilized with a protective sheath to maintained stent in contraction state, this method made the stent more useful and easy delivery in the clinical trials. The biocompatibility, dilating effect of the NiTi stent and the regulation between proliferation and apoptosis of SMC were studied.

Experimental Materials and Methods

The superelastic Ti-50.8at.%Ni alloy self-expanding mesh stent was knitted by 0.15 mm wires, and the outer diameter and the length of the resulting stent was 3.5 mm and 10 mm, respectively. The stent was annealed at 350 °C for 30 min, then cleaned by HF+HNO₃+H₂O solution for 10 seconds, and finally ultrasonically cleaned using deionized water for 3 hours. 50 white big ear rabbits weighing 2.0 kg-3.0 kg were divided into compatibility group (group A: n=36) and atherosclerotic group (group B: n=14). The group A was also subdivided into ten groups according to the sacrificed time: 1 day, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks and 8 weeks. For group B, the rabbits were fed by 15% vitelline, 5% animal oil and 0.5% cholesterol diet. After 7 days, the abdominal aorta was damaged by pulling 3.5 mm PTCA balloon iteratively, and continued to feed oil-enriched diet for 2 months. Then the NiTi self-expanding stent was implanted. Inverse
abdominal aorta angiography was used with 4 ml 76% cardiografin during the procedure and aorta angiography was taken before the rabbit sacrificed, the injected velocity of cardiografin was 4ml/s. The samples for optical microscope, scanning electronic microscope and transmission electron microscope were prepared as routine. The TUNEL detection was followed the assay instruction.

Results and discussion

Six rabbits died in group A because of blood loss by the breakage of femoral, 30 stents were successfully implanted in abdominal aorta in group A and 14 stents in group B. All the animals in which the stent implanted were alive throughout the study. All the stents were widely patented after stent implantation, and remained widely patented throughout the study. There was no detectable migration, dissection, acute closure or sub-acute thrombosis. Rate of patency were 100% both after stent implanted immediately and at the end of animal sacrificed. There was no restenosis in group A. Gross pathologic examinations of rabbits in the group A showed a slight larger vessel in stenting part than adjacent vessels. A thin, translucent, red layer over the stent was observed at 24 hr and a thick white fibrous layer be seen at 72 hr. The fibrous layer covering the stent thoroughly formed a fibrous tube at 72 hr, but it disappeared at 2 weeks after stent implantation. The smooth neointimal surface covered the stent about 70% at 4 weeks. The stent was totally covered by neointima at 6 and 8 weeks, with its surface being smooth and slightly thicker, as typically shown in Fig.1.

The histopathologic examination of neointima at 2 weeks showed a variable orientation of fibers and SMCs, which had covered the NiTi stent wires. At the same time, the NiTi wires impinged on the walls of vessel. More fibers and SMCs were seen at 4 weeks than that of 2 weeks. Inner-elastic fibers layer were fractured and media of the vessels became thinner because of the expansion of the stent wires, the ratio between intima and media was about 1:1. At 8 weeks the stent wires were entirely covered by the neointima containing more SMCs.

TEM results showed that at 2 weeks the endothelial layer was found over the neointima at both side of the wires, but not over the wires. This kind of endothelial cells enriched cell organs, which had a clear entoblast and more endoplasmic reticulum. Neointima was covered by the styloid single endothelial layer thoroughly at 4 weeks. The 8 week sample showed a cube-like hyperplasia type endothelial layer enriched pinocytotic vesicle, and the junction between endothelial cells were tightening, as can be seen from Fig.2.

SEM observations indicated that at 2 weeks the neointima consisted of fibrous tissue and SMCs covered the NiTi wires from the positions the NiTi wires contacted tightly with the vessel wall. At 4 weeks, most of the NiTi wires were covered by neointimal layer. There was an endothelial layer on the neointima surface and tubercle nucleus was found at high magnification. At 8 weeks, the stent was covered by a smooth neointima completely, and the NiTi wires were buried in the vessel wall. An approximately normal endothelial layer was seen on the surface of intima and the tubercle nucleus could be observed either, as shown in Fig.3.

A great amount of foam cells were observed in the intima of group B rabbits, with obvious neointimal hyperplasia being found. Stenotic lesions were detected with angiography, as typically illustrated in Fig.4. The blood fats of pro- and post-stenting were listed in Tab.1. After 60 days of fatty diet and before PTA, 33%±21.82% (N=14) stenosis in the abdominal aorta were developed, and vanished after stent implantation until the rabbits were sacrificed at 8 weeks after stenting. Tab. 2 listed the measured values for the thickness of neointima and media at different groups. It can be suggested that the stimulation of stent was not the main causes promoting atherosclerosis, but atherosclerotic process could be aggravated by the stent implantation.
Fig. 1 Optical microscopic image at 8 weeks. Fig. 2 TEM image at 8 weeks after stent implantation. Magnification was 10 times

Fig. 3 SEM image at 8 weeks. Magnification was 10,000 times

Fig. 4 Optical microscopic image after 60 days diet. Magnification was 4 times

Tab. 1 Results of blood fat changes before and after oil enriched diet for 14 rabbits (** P<0.01)

<table>
<thead>
<tr>
<th>Items</th>
<th>Before oil enriched diet</th>
<th>60 day After diet</th>
<th>8 week after stenting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol [mg/dl]</td>
<td>19.15±8.75</td>
<td>599±281.12**</td>
<td>141.26±63.48**</td>
</tr>
<tr>
<td>High density lipoprotein-c [mg/dl]</td>
<td>12.42±4.90</td>
<td>19.35±8.48</td>
<td>18.49±8.12</td>
</tr>
<tr>
<td>Triglycerin [mg/dl]</td>
<td>53.18±15.42</td>
<td>153.35±57.54**</td>
<td>95.26±29.70**</td>
</tr>
</tbody>
</table>

Tab. 2 Thickness of neointima and media in groups A and B [µm] (* P<0.05)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rabbits</th>
<th>Thickness of neointima between wires</th>
<th>Thickness of neointima under wires to surface</th>
<th>Thickness of media under wires</th>
<th>Thickness of media between wires</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>102.67±7.51</td>
<td>146.67±15.28</td>
<td>65.00±5.00</td>
<td>111.67±7.64</td>
</tr>
<tr>
<td>B</td>
<td>14</td>
<td>116.86±22.16</td>
<td>188.00±23.42</td>
<td>43.21±15.52</td>
<td>87.14±21.64</td>
</tr>
</tbody>
</table>

The observations by electronic microscope showed that some SMCs displayed characteristic alternations in cell morphology. Chromatin condensation and margination showed different stages of apoptosis. The apoptotic cells which had a chromatin condensation and intact cell organs in
morphology were also found, the characteristic alternations were not only detected in the neointimal layer (see Fig. 5), but also in the media layer of stenting components. The TUNEL detection results indicated that most of nucleolus was in the neointima, and positive cell was not detected in the normal vessel. In the group A samples, most of the positive cells appeared around the wires between 3-6 weeks and decreased after 8 weeks. At 8 weeks, a great amount of foam cells were detected in the neointima and the apoptotic cell was mainly detected around the wires. The apoptotic cells were less appeared in areas of foam cells, as illustrated in Fig. 6. Kollum et al [3] identified that the proliferation of SMCs increased significantly after stenting while apoptotic cells increased significantly either.

Fig. 5 TEM image at 2 weeks. Magnification was 20,000 times
Fig. 6 Optical microscopic image at 8 weeks labeled with TUNEL assay, the nucleus of apoptotic cells turned into brown. Magnification was 10 times

Conclusions

All the animals lived throughout the study. There were no detectable migration or dissection of the stents, and there were no acute closures or sub-acute thromboses in the vessels. In group A, the coverage rate of the smooth intima on the stent were about 33%, 70%, and 100% at 2, 4, and 6 to 8 weeks, respectively. Histopathologic examinations of the vessels showed that a variable orientation of fibers and SMCs occurred in the intimae. Moreover, the media of the vessels became thinner due to the expansion of the stent. At 8 weeks, the stent was entirely covered with the intimae, and at these areas the endothelial cells could be found. After 60 days of a fatty diet given to the rabbits, atherosclerosis developed and $33\% \pm 21.82\%$ (n = 14) stenosis existed. High numbers of foam cells could be observed in the intimae of the abdominal aortas. After PTA and stent implantation, the stenoses vanished. The maximum thickness of intima in groups A and B was $146.67 \pm 15.28\mu m$ and $188.00 \pm 23.42\mu m$, respectively. Most of the apoptotic cells were detected not in the normal vessels but rather in the vessels of group A, at 3 to 6 weeks, around the wires, and the numbers of these cells decreased after 8 weeks. Fewer apoptotic cells appeared in the areas of foam cells.

References