

## Clinical Research

# First Experimental Application of Bevacizumab-Eluting PC Coated Stent for Inhibition of Vasa Vasorum of Atherosclerotic Plaque: Angiographic Results in a Rabbit Atheromatic Model

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## Key words:

**Atherosclerosis, drug eluting stent, vasa vasorum, neo-vascularization, bevacizumab, restenosis, coated stent.**

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**Introduction:** Atheromatosis is associated with angiogenesis, through the development of a dense net of vasa vasorum. The role of vascular endothelial growth factor (VEGF) is important in this process. Bevacizumab, an antibody specific for VEGF, has recently been applied in the clinical field. We hypothesized that local delivery of bevacizumab by stent would inhibit the development of vasa vasorum at the stented arterial segment in an atheromatic rabbit model.

**Methods:** We used 10 New Zealand rabbits under atherogenic diet for 3 weeks. We immersed a BiodivYsio stent into a solution of 4 ml bevacizumab as in previous studies. Both eluting stents and non-eluting BiodivYsio stents were implanted in the middle segment of the 2 iliac arteries of the animals, with the same procedural characteristics. Sacrifice following repeat angiogram was scheduled after 28 days.

**Results:** In all animals the procedure of stent loading with bevacizumab and stent implantation was successful. There was no acute or subacute thrombosis. Iliac artery lumen diameters before and immediately after stent placement were similar in all stent treatment groups. At euthanasia stent lumen diameters were similar in all groups. All stents were angiographically patent at the time of euthanasia without aneurysm formation. Moreover, gross pathologic analysis did not show any evidence of vascular necrosis.

**Conclusions:** Bevacizumab-eluting stent implantation in atheromatic rabbit iliac arteries is feasible and safe. This new approach for the treatment of stable and vulnerable plaques needs to be further investigated.

**I**t is known that vasa vasorum make an important contribution to the nutrition of the arterial wall and their impairment alters the arterial structure and function.<sup>1,2</sup> Atheromatosis, however, is associated with angiogenesis, through the development of a dense net of vasa vasorum.<sup>3,4</sup> Intralumen angiogenesis in stable and unstable atherosclerotic plaques increases their vulnerability.<sup>5,6</sup> The role of vascular endothelial growth factor (VEGF) is important in this process, as several studies suggest that VEGF and other angiogenic

factors can promote atherosclerosis by intralumen angiogenesis.<sup>7</sup>

Bevacizumab, an antibody specific for VEGF, has recently been applied in the clinical field for the treatment of metastatic colorectal carcinoma.<sup>8</sup> Until now, there have been no reports concerning the local delivery of bevacizumab by drug-eluting stents or catheter devices to inhibit pathologic neo-vascularization.

We hypothesized that local delivery of bevacizumab by stent would inhibit the development of vasa vasorum at the stent-

ed arterial segment in an atheromatic rabbit model. Thus, the aim of the present experimental study was to assess the feasibility and safety of this approach, and the immediate and long-term angiographic results of the BiodivYsio SV-bevacizumab eluting stent compared to a standard BiodivYsio stent, used in a control group.

## Methods

The experimental protocol was approved by the Institutional Laboratory Care and Use Committee and was conducted in accordance with regulatory guidelines for the care of laboratory animals. We used 10 New Zealand rabbits with average weight  $3.8 \pm 0.4$  kg. All animals consumed an atherogenic diet (0.3% cholesterol and 4.7% coconut oil, Research Diets) for 3 weeks to induce atheroma formation.

The BiodivYsio stent delivery system<sup>9</sup> is coated with phosphorylcholine (PC), a naturally occurring biological substance. The method of impregnating the PC coating involved three steps.<sup>10</sup> The stent was first immersed in a solution of 4 ml bevacizumab (Avastin, 25mg/ml, Roche) for 5 min. After removal from the solution the stent was allowed to dry for 1 min, after which another 10  $\mu$ l of the same solution was pipetted onto the stent. The PC polymer absorbs the solution like a sponge. The stent was again allowed to air-dry for 1 min. This process was then repeated, but with 5 min of air-drying (total preparation time, 12 min).

Both stents were deployed in the middle segment of the 2 iliac arteries through the right carotid artery via a 5F sheath. Eluting and non-eluting stents were 2 mm in diameter and the stent length was 7 mm (2 stents), 10 mm (12 stents) and 18 mm (6 stents). The balloon-expanded stent to artery ratio was intended to be 1.2:1 in all stents. Post-dilatation was required in 12 stents. A final angiogram was performed to confirm the optimal expansion of the stents. All angiograms before and after

the implantation were recorded on videotape. At the end of the procedure, the angioplasty equipment was withdrawn, the carotid artery ligated, and the animal allowed to recover.

The animals remained on an atherogenic diet, and after stent implantation they were treated with aspirin and clopidogrel. At 28 days a follow-up angiogram was performed after accessing the left common carotid artery and then the animals were killed with an intravenous overdose of thiopentone.

## Results

In all animals the procedure of stent loading with bevacizumab and stent implantation was successful and uncomplicated. There were no problems related to the intervention and all interventional devices were deployed successfully. All vessels were angiographically patent at the end of the procedure. There was no acute or subacute thrombosis. All animals survived to 28 days.

Table 1 shows the diameter of the arterial segments at baseline and the balloon-to-artery ratios achieved in each group. Both the arterial diameter and the balloon-to-artery ratios were very similar for each group.

Iliac artery lumen diameters before and immediately after stent placement were similar in all stent treatment groups (Figure 1). At euthanasia stent lumen diameters were similar in all groups. All stents were angiographically patent at the time of euthanasia without aneurysm formation. Moreover, gross pathologic analysis did not show any evidence of vascular necrosis.

## Discussion

The results of the present study showed that bevacizumab-eluting stent implantation in atheromatic rabbit iliac arteries is feasible and safe, without acute and long-term complications.

Recent studies have shown that the development

**Table 1.** Baseline anatomical data and results of stenting in the bevacizumab and control groups.

	Bevacizumab	Control	p value
Artery diameter (mm)	$2.01 \pm 0.02$	$2.02 \pm 0.03$	0.81
Stent length (mm)	$12.1 \pm 4.1$	$12.1 \pm 4.1$	0.99
Balloon/artery ratio	$1.19 \pm 0.01$	$1.19 \pm 0.01$	0.99
MLD after (mm)	$2.21 \pm 0.01$	$2.22 \pm 0.02$	0.87
MLD follow-up (mm)	$2.07 \pm 0.02$	$2.05 \pm 0.02$	0.85

MLD - mean lumen diameter

of vasa vasorum is associated with inflammatory local activation in the atherosclerotic plaques.<sup>4,5,7,11-15</sup> The process of neovascularization in atheromatosis is mainly mediated by VEGF.<sup>16-19</sup> Thus, inhibition of VEGF may eliminate plaque neovascularization and the consequent development of high-risk atheromatic plaque.

Bevacizumab is a specific antibody against VEGF and is currently used in clinical practice, as a potent antiangiogenic agent.<sup>8,20-22</sup> We postulated that local delivery of bevacizumab, delivered by a dedicated stent, could inhibit plaque neovascularization. The aim of the current study was to investigate the feasibility and safety of this approach in a rabbit atheromatic model.

Indeed, we did not observe any adverse event, such as acute or subacute thrombosis. Late thrombosis was not evident in any animal. Despite the lack of data regarding the thrombogenicity of the device, we selected the long-term combined antiplatelet therapy in this first series of experiments, as in the era of drug-eluting stents this is the usual approach in clinical practice. Future experimental studies will be required to investigate the appropriate time for dual antiplatelet treatment.

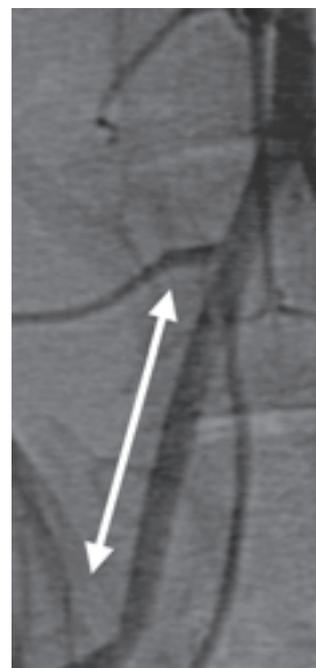
The safety of the bevacizumab-eluting stent was also confirmed by gross observation of the specimens. There were no signs of necrosis and all stented segments were patent. Although the gross specimen observation and the angiographic results provide evidence for the safety of this new stent, a histological examination is needed to confirm these preliminary observations.

## Conclusion

The preliminary results of the present study showed that bevacizumab-eluting stent implantation in atheromatic rabbit iliac arteries is feasible and safe, and the immediate and late angiographic results demonstrated that there is no increased thrombogenicity compared to the control group. Histological analysis will demonstrate the effect of bevacizumab-eluting stents on neovascularization, and will determine whether a clinical study will be justified. This new approach to the treatment of stable and vulnerable plaques needs to be further investigated.

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**Figure 1.** Follow-up angiogram of right iliac artery 28 days after bevacizumab-eluting stent implantation (arrow indicates the length of the stent). The stented segment is patent, without any sign of thrombosis or intimal hyperplasia.

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