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Restenosis is not associated with stent length in a pig model of coronary stent implantation

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Abstract

Background: *The aim of this study was to determine if stent length is by itself a risk factor for intimal proliferation and restenosis. Long lesions represent an independent risk factor for restenosis after coronary stent implantation. A longer stented segment might result in a higher probability of restenosis.*

Methods: *Twenty-two 7-month-old male farm pigs underwent implantation of two steel stents, one short (8 mm length) and one long (16 mm length), in the right coronary artery. The pigs were sacrificed 28 days after stent implantation and histomorphometric analysis of the coronary arteries was performed for neointimal area proliferation and area stenosis evaluation.*

Results: *Seventeen short stents and 19 long stents were finally implanted. There were no differences in neointimal proliferation (1.84 ± 0.64 mm^2* vs. *1.81 ± 0.94 mm^2, p = 0.84), area stenosis (40 ± 9%* vs. *41 ± 19%, p = 0.86) and lumen area (2.96 ± 1.30 mm^2* vs. *2.51 ± ± 1.18 mm^2, p = 0.21) between the short stent group and the long stent group, respectively.*

Conclusions: *These data suggest that stent length by itself does not influence restenosis extent in the porcine model.* (Cardiol J 2008; 15: 458–462)

Key words: restenosis, angioplasty, stent

Introduction

Coronary angioplasty has been established as an efficacious and safe procedure for alleviating myocardial ischemia associated with coronary artery atherosclerosis. However, the major factor limiting the long-term success of the procedure is restenosis of the dilated segment, which happens in approximately 20–40% of patients, in the pre-coated stent era.

Animal studies suggest that post-angioplasty restenosis results from platelet adhesion to the area of endothelial damage at the dilation site, with subsequent release of potent smooth muscle cell constrictors, mitogens and various growth factors [1]. Although clinical and angiographic restenosis rates in selected lesions are reduced with coronary stenting as compared to angioplasty, it is not known whether this benefit extends to other types of lesions. Multiple or long coronary stents are now being implanted in long lesions or in tandem lesions. Previous studies [2–4] have shown higher restenosis rates with multiple overlapping stents. However, it is difficult to assess the relative importance of increased metal exposure with multiple partial overlapping stents versus a long lesion or a long

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stented segment. A longer stented segment might result in a higher probability of restenosis. However, there is little information on the relation between stented segment length and restenosis, and the information from the present literature is controversial. Some works [5–7] show that stented length is a predictor of restenosis, whereas others give the opposite results: that there is no such relation [2, 3]. Most of these works were performed with multiple overlapping stents. The higher restenosis rate was explained by greater metal density with multiple overlapping stents, which results in more pronounced neointimal hyperplasia [8].

Therefore, the aim of the present study was to determine if stent length is by itself, and not because of coinciding long lesion, a predictor for intimal proliferation and restenosis in the pig model of in-stent restenosis.

Methods

Animals, treatment and study design

The Athens Animal-care Committee approved all protocols. The care of the animals complied with the guidelines of the European Community. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institute of Health (Publication No. 85-23, revised 1996).

Twenty-three male farm-pigs, 7 months old, weighing 20–25 kg were catheterized, and one or two stainless steel stents were implanted in the right coronary artery of each pig. The pigs were allowed free access to fresh water ad libitum and were fed regular pig chow.

On the morning of the procedure, pre-anesthetic medication and atropine were administered by a single intramuscular injection in the large muscle of the caudal thigh. As soon as the trachea was intubated with a 6.5–7.0 mm cuffed tracheal tube, anesthesia was induced with propofol IV, followed by fentanyl and pancuronium bromide for analgesia and neuromuscular blockade, respectively. Ventilation was controlled using intermittent positive pressure. Oxygen-enriched air was administered to maintain a PaO_2 greater than 100 mm Hg, and the ventilatory settings were adjusted to maintain normocarbia. Anesthesia and analgesia were maintained by continuous IV infusions of propofol and fentanyl. Neuromuscular blocking was maintained by intermittent doses of pancuronium. The anesthetic, analgesic, and neuromuscular blocking drips were stopped about 30 min before the operation ended, and neostigmine (1–2 mg) together with atropine (0.5 mg) were administered IV to reverse the residual neuromuscular blockade at the end of the operation. Other monitoring included continuous ECG, pulse oximetry and expiratory PCO_2.

The animals received 5000 IU of heparin and 200 mg lidocaine by intra-arterial injection, and a guiding catheter was introduced via the sheath and advanced over a guidewire to the right coronary artery. We chose the right coronary artery in order to avoid the natural tapering of the coronary arteries that would be expected to result in greater overstretch in stents of equal diameter placed more distally; this, in other instances, could affect the results of the experiment if one of the two lengths was consistently deployed more distally.

After angioplasty and stent placement, the animals were allowed to recover and gained weight steadily.

Intracoronary glyceryl trinitrite (200 μg) was injected, and then angiograms were recorded in the 45° left anterior oblique projection using hand injections of 10 mL contrast medium. Stent implantation was performed using appropriately sized non-compliant balloons inflated to 10 atmospheres 3 times, for an inflation time of 30 s. The stents were crimped on 20 mm balloon catheters of the appropriate size (10–30% greater than the vessel diameter, to create arterial injury and thus ensure a measurable neointimal response) and were implanted using standard angioplasty guide wires. Final angiograms were recorded after a repeat injection of 200 mg glyceryl trinitrite.

In all animals, treatment with two stents, one short and one long, was attempted in random order (long-short, short-long). The distance between the two stents was more than 2 cm. However, unfavourable anatomy or periprocedural difficulties might alter the course of the experimental protocol and only one stent could be delivered.

All the stents had the same strut thickness and the same geometric patterns.

After coronary catheterization the introducer sheath was removed and the femoral artery ligated. The muscle and skin was closed using appropriately sized absorbable suture material. The pigs were recovered and monitored in sternal recumbency, and then returned to routine care.

Euthanasia

On the 28th day after stent implantation, as determined by the study protocol, euthanasia and tissue harvesting were performed. The animals were sedated as in the first study. Then they were catheterised from the other femoral artery and

Figure 1. Digital micrograph of section from pig coronary artery harvested 4 weeks after stent implantation, to be used for histomorphometric assessment.

a diagnostic angiography was performed after heparin 5000 IU was given intrasheath.

After 2–5 min the pigs were killed by a high dose of KCL. The heart was rapidly excised by medial sternotomy and the aorta canulated. The vasculature was cleared of blood using 0.9% NaCl, then perfusion fixed at 100–110 mm Hg pressure for 10–15 min using neutral buffered (pH 7.0–7 4) formalin 10%. The heart was then immersed in the same fixative for an additional 24–48 hours before further processing.

Coronary segments containing stents, with a 1-cm normal segment proximal and distal to the stented site, were carefully trimmed from the hearts with adventitia and some perivascular adipose tissue, but not myocardium. They were dehydrated through graded ethanol series 70%, 80%, 95% and 100%, exchanged with acetone and embedded in methacrylate resin. One hundred cross-sections fifty μm in thickness (~6 per artery) were cut using a diamond-edged, low-speed saw, ground to ~60––l00 μm and polished free of surface scratches.

Each section was evaluated and measured by two experienced observers unaware of the treatment group assignment. Assessment of the stented coronary arteries included estimation of the extent of vessel injury and healing response, luminal and mural thrombosis, intramural hemorrhage, inflammatory infiltrates, necrosis, mineralization and arterial wall fibrosis. For stented arteries (Fig. 1), each coronary artery segment showing evidence of stent injury (presence of stent cross-section or its imprint left by artefact loss) was measured for the following variables: luminal area, internal elastic lamina area (with tracing remaining at the adluminal aspect of the stent profiles), external elastic lamina area and maximal intimal thickness. Additionally, the extent of injury at each stent profile site was estimated using the following grading scale: 0 — stent not in contact with arterial media; 1 — stent in contact with media, internal elastic lamina is intact; 2 — stent in contact or within media, internal elastic lamina broken; 3 — stent within media and contacting external elastic lamina; and 4 — stent within tunica adventitia. A mean stent injury score for each section was also determined.

Histomorphometry was performed using computer-assisted planimetry. Analog signals from a Hitachi 3-chip CCD video camera coupled to a Nikon Eclipse E600 or equivalent light microscope at 20X magnification were captured and digitized with a Scion capture board coupled to an HP Vectra 266 MHz or equivalent PC. Digitized images were measured using Image Pro Plus software by tracing with a CalComp 12" electronic tracing tablet. Instrument calibration was verified prior to each measurement session. Comments regarding the appearance of each segment were also recorded. The average of each measurement of all segments of each artery was then calculated to obtain the vessel averages, which constituted the final sample sizes.

When branch vessel ostia are present in the section these were noted. When they were small (diameter < 1/3 of the primary vessel diameter), the tracings spanned the ostia rather than following the luminal and tissue plane contours. When they were large (diameter > 1/3 of the primary vessel diameter), the segment was not included for measurement. In the case of organized intramural hemorrhage or mural thrombus, such zones were included in the appropriate area of measurement (e.g. adventitia, neointima) and their presence was recorded.

The data were then grouped according to treatment assignment. A mean value of the appropriately injured sections for each artery was calculated and the resultant values constituted the final sample sizes. In the stented vessels, luminal area, vessel area (external lamina), intimal area, maximal intimal thickness, injury score and intimal area/injury score were assessed in order to investigate the effects of the short versus long stents using unpaired one-tailed Student t-tests. Linear regression analysis using the least square difference was used in order to examine possible correlations between injury score and neointimal area. A confidence level of 95% was used to test the null hypothesis.

Table 1. Morphometric analysis at four weeks after stent implantation.

	Short stents (17 arteries)	Long stents (19 arteries)	P
Lumen area [mm^2]	2.96 ± 1.30	2.51 ± 1.18	0.21
Neointimal area* [mm^2]	1.84 ± 0.64	1.81 ± 0.94	0.84
Media area [mm^2]	1.28 ± 0.46	1.49 ± 0.65	0.54
Neointimal thickness at stent struts* [mm]	0.26 ± 0.07	0.22 ± 0.03	0.76
Media thickness at stent struts [mm]	0.17 ± 0.03	0.17 ± 0.02	0.92
Neointimal thickness between stent struts [mm]	0.24 ± 0.08	0.21 ± 0.03	0.65
Media thickness between stent struts [mm]	0.19 ± 0.03	0.18 ± 0.02	0.83
External area [mm^2]	4.68 ± 1.60	4.61 ± 0.87	0.76
Area stenosis (%)	40 ± 9	41 ± 19	0.86
Injury scale	1.72 ± 0.50	1.42 ± 0.42	0.43
Balloon/artery	1.33 ± 0.20	1.22 ± 0.16	0.37

*including area occupied by the stent strut

Results

Twenty-two animals were enrolled in this study. One animal died because of artery dissection after stent implantation. Seventeen short stents and 19 long stents were successfully implanted in the animals right coronary arteries.

No changes in heart rate, electrocardiographic variables or blood pressure were observed during angioplasty.

The balloon/artery ratio and artery diameter were similar in the two groups (Table 1).

Lumen area, neointimal area and media area, as well as the other markers of intimal proliferation such as the neointimal thickness at the stent struts and between struts, and media thickness at and between stent struts, were similar in the two studied groups (Table 1). The stenosis area and external area were also similar. The order in which the stents were placed (long-short, short-long) did not influence the results.

The injury score was the same in the two groups. The injury scale was correlated to the neointimal thickness at stent struts ($r = 0.46$, $p = 0.02$), and to the neointimal thickness between stent struts ($r = 0.45$, $p = 0.03$), in the whole group of studied arteries (Fig. 2).

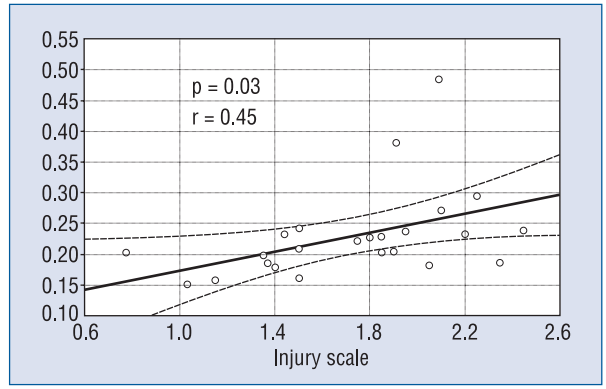

Figure 2. The correlation between the injury scale and the neointimal thickness between stent struts in the whole group of studied arteries.

Discussion

In the present study, bare steel stents were deployed in an oversized manner to promote neointimal hyperplasia, resulting in significant vessel injury. Intimal proliferation area and area stenosis were independent of the length of stent.

Kobayashi et al. [5] examined, retrospectively, the restenosis rate in patients who underwent stenting and divided them in 3 groups according to the length of the stented segment. They demonstrated that a longer stented segment is an independent predictor of restenosis without an influence on the risk of subacute thrombosis. This work had the drawback that they used different types of stents and in a large proportion they used multiple stents in one lesion. Accordingly, Schomig et al. [6] showed that lesion length was an independent correlate to the amount of late lumen loss. The same group of investigators [9] showed, in a retrospective study, that lesion length was an independent risk factor for restenosis after stenting coronary arteries. The risk was further increased by multiple stent placement and overlapping stents that were

also independent risk factors of restenosis. Folley et al. [7], in a prospective study, demonstrated that, using elective Magic Wallstent implantation, stent length was independently associated with greater angiographic restenosis. Recently, however, Pan et al. [10] demonstrated that patients with diffuse lesions treated by single long stents did not have a better late outcome than those who received multiple stents.

In contrast, Strauss et al. [3], after implantation of Wallstents, and Ellis et al. [2] showed that there was no relation between stent length and restenosis. Ellis et al. [2] demonstrated that the incidence of restenosis was higher at sites where multiple overlapping stents were placed. The higher restenosis rate was explained by greater metal density with multiple overlapping stents, which results in more pronounced neointimal hyperplasia.

It is possible, however, that the relation between stent length and restenosis that occurred could be the result not of the stent itself but more of the lesion length than that of the stent length.

The present study was prospective; we used one type of stent, there were no different lengths of lesion, and no lesions at all, in the two groups. We selected the 28-day follow up period because there is literature supporting this time period as sufficiently long to allow for sensitive detection of restenosis [11].

The fact that the pigs had no native lesions is a possible limitation of the study, but on the other hand it was a necessity in order not to have differences in lesion length. The present study has to be extended to answer the question of whether stent length is by itself a risk factor for restenosis in coated stents.

Conclusions

The present study showed that stent length gives no prediction for in-stent restenosis in the pig model.

Acknowledgements

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