**Effect of a novel drug-eluted balloon coated with genistein before stent implantation in porcine coronary arteries**

*[Effect of a novel drug-eluted balloon coated with genistein before stent implantation in porcine coronary arteries.Sheiban I, Anselmino M, Moretti C, Biondi-Zoccai G, Galloni M, Vignolini C, Mattoni M, Sciuto F, Omedè P, Trevi GP. Clin Res Cardiol. 2008 Dec;97(12):891-8. doi: 10.1007/s00392-008-0705-2.]*

# EFFECT OF A NOVEL DRUG-ELUTED BALLOON COATED WITH GENISTEIN BEFORE STENT IMPLANTATION IN PORCINE CORONARY ARTERIES

Imad Sheiban, Matteo Anselmino, Claudio Moretti, Giuseppe Biondi-Zoccai, Marco Galloni*, Cristina Vignolini*, Mario Mattoni*, Filippo Sciuto, Pierluigi Omedè, Gian Paolo Trevi.

Division of Cardiology, University of Turin, Turin, Italy

*Division of Animal Pathology, University of Turin, Turin, Italy

**Conflicts of interest:** none

**Funding:** supported by an unrestricted grant by Sahajanand Medical Technologies Pvt. Ltd

**Word count:** 3540**,** including text, figure legends, and references

**Key-words:** developmental biology; angioplasty; Genistein; restenosis.

**Corresponding author:**

Prof. Imad Sheiban, Interventional Cardiology, Division of Cardiology,

University of Turin, San Giovanni Battista Hospital, Turin, Italy.

Phone: +39-011-6334446. Fax: +39-0116967053. Email: isheiban@yahoo.com

Background. The major drawback of stent implantation in native human coronary vessels is the occurrence of restenosis. Drug-eluting stents significantly reduce restenosis after percutaneous coronary intervention (PCI), but may be associated with persistent local inflammation involved in the restenosis mechanisms. In this setting coating coronary devices with anti-inflammatory agents represents an intriguing alternative to stent-based local drug delivery. The aim of the present study was to test in a porcine model the safety and efficacy of a novel Genistein-eluting balloon preceding coronary stenting.

Design. Female piglets underwent PCI in a randomized fashion with either a Genistein-eluting or a standard balloon angioplasty, followed in all vessels by bare-metal stent implantation. Pigs were sacrificed at different time points to appraise safety (i.e. endothelialization) and efficacy (i.e. anti-inflammatory and anti-proliferative effects): 1, 4, and 6-8 weeks following PCI.

Results. Overall analysis was conducted on 14 piglets. Twenty-five bare-metal stents were implanted preceded by angioplasty with a conventional balloon in 13 vessels and by the Genistein-eluted balloon in 12. No untoward effects were reported in either group. Healing and endothelialization appeared universal within four weeks. The Genistein-eluted balloon group disclosed a significant reduction, at four weeks from implantation, of the peri-stent inflammatory cells count (mononucleocytes 39±32 vs. 96±29 per square millimetre, p=0.019. This effect did not clearly translate into a trend towards a reduced neointimal hyperplasia at six-eight weeks (0.13±0.11 vs. 0.14±0.09, p=0.835).

Conclusion. This study provides the first *in vivo* demonstration of the anti-inflammatory effects of a Genistein-eluting balloon in PCI, warranting further research including the combination of a Genistein-eluting balloon with standard drug-eluting stent.

## Introduction

The major drawback of stent implantation in native human coronary vessels is the occurrence of restenosis, especially in complex lesion subsets such as diffuse disease and small vessels. As stents resist arterial remodelling (1-4), restenosis after stent implantation is principally due to neointimal hyperplasia. Although superior to angioplasty alone, the restenosis rates after bare-metal stent implantation are as high as 20% to 40% at 6 months. Given this situation the medical community devoted, in the latest years, great efforts to perceive alternative approaches to prevent restenosis (5,6). Systemic antiproliferative agents (7), coronary brachytherapy (8), contrast mediumï antiproliferative formulations (9) have been tested. None has contrasted the central role of drug-eluting stents (10,11,12) although their long-term results seemingly limited by the delay in healing related to the risk of late stent thrombosis and hypersensitivity reactions (13,14,15).

Inflammation plays a relevant role among the atherosclerosis and restenosis mechanisms, thus the attempt of reducing inflammatory signalling holds the potential to attenuate restenosis without significantly delaying arterial healing. Recently, although the use of the oral corticosteroid prednisone following bare-metal stent deployment has yielded interesting results (16) a dexamethasone-eluting stent has failed to prove effective (17). In this setting an angioplasty balloon coated with Genistein, an officially approved and clinically safe flavonoid anti-inflammatory agent not presenting major cardiovascular side effects or common intolerances may represent the most advanced and possibly superior alternative.

The aim of the present study was to test in a porcine model the safety and efficacy of a novel Genistein-eluting (18) balloon preceding coronary stenting. Endothelialization, anti-inflammatory and anti-proliferative effects were appraised at different time points and compared to controls in which the coronary stenting was preceded by conventional angioplasty balloon.

## Materials and methods

*Study design*

Cross-bred young adult female piglets weighting between 35 and 60 kilograms were investigated conforming to *The Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and current practice guidelines. Moreover, the study was approved by the local regulatory committee for animal welfare. Two vessel samples within the left circumflex, the left anterior descending and the right coronary artery were selected from each animal, unless these targets were small (eg non-dominant right coronary arteries) or challenging for stenting (eg tortuosity or anomalous take-off). The selected coronary arteries were then compared as tests and controls within the same or separate animals. The decision whether to treat the target vessel with a conventional or the Genistein-eluted angioplasty balloon was based on computer-generated random numbers without blocks.

The drug-eluting balloon (Sahajanand Medical Technologies Pvt. Ltd., Gujarat, India) constitutes of a biodegradable polymeric matrix (Poly Lactide-co-Glycolide) coated with Genistein (0.7 µg/mm2, 112.1 µg on each 3.0 * 17 mm balloon). During the procedures related to the delivery of the coated balloon catheter 15-20% of the drug is released (within 30 to 40 seconds) in the blood stream; the remaining 60-70% of the drug is intended to reach the local target area (product label data).

In all target vessels the angioplasty was followed by a standard balloon-expandable bare-metal stent implantation. The animals were clinically observed every day until one week postoperatively and then weekly by a board certified veterinarian. In case of any abnormality, a complete veterinary clinical examination and recording was realized. The piglets were sacrificed at one (3 treated segments), four (10 treated segments), and within six and eight weeks (12 treated segments) following stent implantation (Figure 1). Coronary segments (1 allocated to conventional and 2 allocated to Genistein-eluted angioplasty balloon) unsuitable for stenting or from piglets dying before the pre-specified time point were excluded.

*Coronary angioplasty and stenting deployment*

The animals were given Atropine sulphate, Xylaxine, and Ketamine and subsequent anaesthesia was induced by Thiopentone sodium. Following orotracheal intubation, on ventral recumbence, they were given $O_2$-$N_2O$ (1:1) and Halothane 1.5% to 2%. Pancuronium bromide was used as muscle relaxant. The ventral neck area was then clipped, scrubbed with Povidone soap and prepared with Povidone iodine solution based on standard aseptic techniques. Subsequently a 7 French introducer sheath was inserted in the common carotid artery, Amplatz 7 or 6 French left coronary catheters were engaged into the respective coronaries under full heparinisation (3 mg/Kg of body weight). The coronary arteries were imaged by a standard angiographic technique and the target vessel diameters estimated by comparison with the angiographic catheter. The conventional or Genistein-eluted angioplasty balloons and the bare-metal stents were delivered using the delivery catheters provided by the respective manufacturer. Balloon inflations were performed at nominal pressure for 40-50 seconds in both groups. The angioplasty was in all cases followed by a standard balloon expandable bare-metal stent implantation. Over-stretching ratio was limited within 1:1 and 1:3 balloon-diameter ratio, appraised by visual estimation of reference vessel diameter matched with the appropriately sized balloon at nominal dilation pressure. The surgical wound was then closed and dressed as per standard technique. Analgesic and antibiotic coverage was provided for the five following postoperative days. Antiplatelet and anticoagulant therapy was prescribed as listed: clopidogrel hydrochloride 75mg plus aspirin 75mg once the day, starting two days before the procedure and maintained up to the end of the study; heparin sodium 3mg/Kg of body weight IV bolus started during the implantation and not reversed post surgically; two doses of Fraxiparine (0.4ml each) subcutaneously at 12 hours interval post implantation until study end.

*Qualitative and quantitative coronary analysis*

The animals were euthanized by an excess dose of intravenous thiopentone sodium following a standard angiographic examination of the target vessels. The gross specimens consisted of the heart with the stents *in situ*. The heart was perfused with formalin 4% for 20 minutes and consequently fixed in methyl Carnoy's solution (60%  methanol, 30% clorophormium and 10% glacial acetic acid) for 24 hours and in pure ethanol for the next 24 hours. The entire segment of the blood vessel with the stent was cut at either end from the host vessel and processed. Final inclusion was performed the following morning fixing the specimens pretreated in a clorophormium solution for two hours in formalin melted at 70°C over night. One hundred μm thick sections were cut perpendicular to the long axis of the vessel, at the proximal, mid and distal stent level. The sections were stained with toulidine blue/haematoxylin and eosin/VVG. In addition, 10 mm thick sections of proximal and distal segments (5 mm from the stent edge) were processed and embedded in paraffin wax and 5 μm thick sections were cut and stained with haematoxylin and eosin. Relevant photomicrographs were taken for each segment of interest.

Histomorphometric variables of the target vessels were analyzed by the NIH Image Program (PC version Scion Image, SCION Corp). Patency was evaluated as the degree of in-stent stenosis by blinded histomorphometry and acute and chronic tissue response by blinded graded histopathology. The evaluated parameters were equivalent luminal diameter, neointimal thickness, percentage of lumen stenosis, total and mononucleocytes cellularity count. Furthermore injury and inflammation scores were appraised as described by Schwartz et al. (19) and Kornowski et al. (20) respectively. Discrepancies were resolved by mutual consensus.

*Statistical analysis*

Histomorphometric variables of the proximal, mid and distal cross-sectional planes are reported as mean values per stent. Continuous variables, presented as means and standard deviations, were compared by Studentös *t* test for equality of means after a normalized distribution was assured. Box and whisker plots were computed to visualize medians and lower and higher quartiles. Categorical variables, presented as counts and percentages, were compared in cross tabulations tables by means of the Pearson chi-square test and likelihood ratio. The level of significance was taken as two-tailed p=0.05. All statistical analysis was performed with SPSS 11.5 (SPSS Inc).

**Results**

Overall analysis was conducted on 14 piglets. Twenty-five bare-metal stents were implanted preceded by angioplasty with a conventional balloon in 13 vessels and by the Genistein-eluted balloon in 12 (Figure 1). None of the piglets presented clinical abnormalities during the study protocol.

No untoward effects were reported comparing the histomorphometric and histopathologic measures of the vessels treated with Genistein-eluting (n=2) or conventional angioplasty balloon (n=1) one week after implantation (neointimal thickness 0.03 vs. 0.03, p=0.963; percentage of lumen stenosis 6.25 vs. 5.43, p=0.550; mononucleocytes cellularity 261.0 vs. 271.0, p=0.976; injury score 0 in both groups).

Ten target vessels were explanted at four weeks from implantation. Photomicrographs of the specimens at this time point disclosed universal healing and endothelialization in the stent struts (Figure 2). The histomorphometric and histopathologic measures comparing the target vessels treated with the Genistein-eluted balloon (n=4) with those treated with a conventional balloon (n=6) are reported in Table 1. Box and whisker plots are visualized in Figure 3. The Genistein-eluted balloon group disclosed a significant reduction, at four weeks from implantation, of the peri-stent inflammatory cells count (mononucleocytes 39±32 vs. 96±29 per square millimetre, p=0.019; Figure 4).

The remaining 12 target vessels were explanted from the piglets within six and eight weeks after the implantation. The histomorphometric and histopathologic measures comparing the vessels treated with Genistein-eluted (n=6) or conventional angioplasty balloon (n=6) are reported in Table 2. Box and whisker plots are visualized in Figure 5. The overall trends were towards a reduction of neointimal hyperplasia, of percentage of lumen stenosis, of the injury scores and of the count of inflammatory cells, without reaching nominal statistical significances.

**Discussion**

This study provides the first *in vivo* demonstration of the anti-inflammatory effect of a novel Genistein-eluting balloon. In this porcine coronary artery model the tested device provided consistent data on safety and biofunctional efficacy.

The coronary arteries of piglets have previously proved to respond in a similar manner to the human coronary arteries after injuries (21,22). In fact the in-stent neointimal thickness, usually caused within 28 days from an injury, has been described as identical to the human restenotic neointima. The direct proportional correlation within this neointimal thickening and the degree of the injury has been an extremely relevant finding, permitting the creation of an injury-response regression relationship that quantities the response to the potential therapies (23). Given this evidence, the porcine coronary models using injuries caused by either stenting or overstretching alone are now accepted standards, by which potential restenosis therapies are studied.

Despite the widespread use of intracoronary stents (20), in-stent restenosis remains a major clinical problem. In the attempt of achieving the ideal balance between healing and suppression of neointimal hyperplasia previous animal studies (19,25) have established a significant correlation between the degree of arterial injury caused by the metallic wire coils and the resultant neointimal thickness and lumen stenosis at the stented site. The restenosis and occlusion after initially successful percutaneous procedures seem to be, in a large extent, due to the excessive formation of neointimal tissue in response to the unavoidable injury that occurs during balloon dilation and stent implantation. This process continues for weeks and months may finally result in the occlusion of the artery lumen. The mechanism driving this process after the initial phase of wound healing is yet not completely clear (26). In coronary arteries, however, excessive formation of neointima has been successfully inhibited by the implantation of drug-eluting stents, providing a platform for sustained drug release, which is believed to be a precondition of successful restenosis inhibition (27). Their implementation in clinical practice has however disclosed unexpected long-term results due to the delay in healing accompanied by the risk of late stent thrombosis (28,29).

Few alternative methods have been tested to distribute specific drugs at the vessel wall sites. An angiographic contrast medium enriched with paclitaxel was found to be associated with an inhibition of neointimal proliferation four weeks after the intervention (30,31). Another interesting approach derives from the possibility of coating with antiproliferative drugs the balloons for angioplasty (32-34).

On the other hand the inflammation, with subsequent release of chemotactic and growth factors after arterial injury, has been raised as one of the major contributing mechanisms of the restenosis (35,36). Despite the paucity of data indicating either causality or correlation between the degree of inflammation after arterial injury and the amount of neointimal formation Kornowski et al.(20) have delineated the role of inflammation in the neointimal formation within stents, with vast potential therapeutic implications. Indeed interventions focused on reducing inflammatory signalling carry the promise of attenuating restenosis without significantly delaying arterial healing. Only recently, the use of the oral corticosteroid prednisone after bare-metal stent deployment has yielded significant reductions in restenosis (16,37). Although these interesting results with oral corticosteroids following bare-metal stent deployment a large, multicentre analysis of a dexamethasone-eluting stent in patients with acute coronary syndromes offered a low rate of clinical events at six months, but no anti-restenosis effect (17).

Given these presumptions we aimed to evaluate the effects of an anti-inflammatory drug-eluting balloon. The choice of the coated drug, Genistein, was based on the need of an officially approved and clinically safe agent, not presenting major cardiovascular side effects or common intolerances. Genistein, a phytoestrogen resembling 17β-estradiol, has been typically prescribed in women based on its ability, besides the anti-inflammatory effect, to positively regulate bone cell metabolism without harmful estrogenic activity in the breast and uterus (38). Furthermore in experimental studies Genistein has proved the ability to inhibit collagen-induced platelet aggregation, enhance NO production from the endothelium, decrease cell apoptosis, and inhibit neointima formation, proliferation and migration of vascular smooth muscular cells (39,40).

The Genistein-eluting balloon proved safe. No untoward effects were found in any of the animals, including those sacrificed as early as one week post-PCI. An even more relevant finding was the universal healing and endothelialization in the stent struts within four weeks.

Even more the tested device proved biological efficacy. The target coronary vessels treated with the Genistein-eluting balloon disclosed, at four weeks, a significant reduction in peri-stent inflammatory cells. This completely quantitative variable is certainly more reliable compared to the inflammatory score, especially in small sample populations as animal studies are. The lack of maintained evident anti-inflammatory effectiveness in the animals sacrificed within six and eight weeks does not surprise. As recently standardized, inflammation and healing in pigs coronary arteries suggests a time comparability of approximately 1 to 6 porcine to human, with pigs healing more rapidly (19). For this reason the inflammatory process may, at this timepoint, be completely resolved. Furthermore the progressive elution of the drug may be close to finalized six-eight weeks from the implantation.

In the clinical setting, the Genistein-eluting balloon holds the potential to limit inflammation and enhance earlier strut endothelialization following stenting. Further studies exploring the synergy of Genistein-eluting balloon followed by drug-eluting stent implantation are thus warranted.

In conclusion, this study is the first *in vivo* demonstration of the anti-inflammatory effects of a novel Genistein-eluting balloon, providing consistent data on safety and biofunctional efficacy of this device in a porcine coronary artery model. Local delivery of anti-inflammatory drugs before implantation of stents might indeed yield significant clinical benefits among patients with coronary artery disease.

**Funding**

Sahajanand Medical Technologies Pvt. Ltd (unrestricted grant to IS).

**Conflict of Interest**

None declared.

**References**


1. Schatz RA, Palmaz JC, Tio F, Garcia F, Garcia O, Reuter SR. Balloon expandable intracoronary stents in the adult dog. Circulation 1987; 76:450-457.

2. Hoffman R, Mintz GS, Dussaillant GR, et al. Patterns and mechanisms of instent restenosis: a serial intravascular ultrasound study. Circulation 1996; 94:1247-1254.

3. Dussaillant GR, Mintz GS, Pichard A, et al. Small stent size and intimal hyperplasia contribute to restenosis: a volumetric intravascular ultrasound analysis. J Am Coll Cardiol 1995; 26:720-724.

4. Gordon PC, Gibson M, Cohen DJ, Carroza JP, Kuntz RE, Baim DS. Mechanisms of restenosis and redilation within coronary stents-quantitative angiographic assessment. J Am Coll Cardiol 1993; 21:1166-1174.

5. Beier F, Gyöngyösi M, Raeder T, von Eckardstein-Thumb E, Sperker W, Albrecht P, Spes C, Glogar D, Mudra H. First in-human randomized comparison of an anodized niobium stent versus a standard stainless steel stent - an intravascular ultrasound and angiographic two-center study: the VELA study.Clin Res Cardiol 2006; 95(9):455-60.
.
6. Wöhrle J, Nusser T, Kestler HA, Kochs M, Hombach V. Comparison of the slow-release polymer-based paclitaxel-eluting Taxus-Express stent with the bare-metal Express stent for saphenous vein graft interventions. Clin Res Cardiol 2007; 96(2):70-6.

7. Lincoff AM, Topol EJ, Ellis SG. Local drug delivery for the prevention of restenosis: fact, fancy, and future. Circulation 1994; 90:2070-2082.

8. Kuntz RE, Baim DS. Prevention of coronary restenosis: the evolving evidence base for radiation therapy. Circulation 2000; 101:2130-2133.

9. Scheller B, Speckb U, Romeikec B, Schmitta A, Sovakd M, Böhma M, Stolla HP. Contrast media as carriers for local drug delivery. Successful inhibition of neointimal proliferation in the porcine coronary stent model. Eur Heart J 2003; 24:1462-1467.

10. Moses JW, Leon MB, Popma JJ, et al. Sirolimus-eluting stents versus standard stents in patients with stenosis in a native coronary artery. N Engl J Med 2003; 349:1315-1323.

11. Park SJ, Shim WH, Ho DS, et al. A paclitaxel-eluting stent for the prevention of coronary restenosis. N Engl J Med 2003; 348:1537-1545.
.
12. Zahn R, Hamm CW, Schneider S, Zeymer U, Richardt G, Kelm M, Levenson B, Bonzel T, Tebbe U, Sabin G, Nienaber CA, Pfannebecker T, Senges J. The Sirolimus-eluting coronary stent in daily routine practice in Germany: Trends in indications over the years. Results from the prospective multi-centre German Cypher Stent Registry. Clin Res Cardiol 2007; 96(8):548-556.

13. Virmani R, Kolodgie FD, Farb A, Lafont A. Drug eluting stents: are human and animal studies comparable? Heart 2003; 89:133-138.

14. Lowe HC, Schwartz RS, Mac Neill BD. The porcine coronary model of in-stent restenosis: current status in the era of drug-eluting stents. Catheter Cardiovasc Interv 2003; 60:515-523.

15. Carlsson J, von Wagenheim B, Linder R, Anwari TM, Qvist J, Petersson I, Magounakis T, Lagerqvist B. Is late stent thrombosis in drug-eluting stents a real clinical issue? A single-center experience and review of the literature. Clin Res Cardiol 2007; 96(2):86-93.

16. Versaci F, Gaspardone A, Tomai F, et al. Immunosuppressive therapy for the prevention of restenosis after coronary artery stent implantation (IMPRESS study). J Am Coll Cardiol 2002; 40:1935-1942.

17. Ribichini F, Tomai F, Paloscia L, Di Sciascio G, Carosio G, Romano M, Verna E, Galli M, Tamburino C, De Cesare M, Pirisi R, Piscione F, Lanteri G, Ferrero V, Vassanelli C. Steroid-eluting stents in patients with acute coronary syndrome: the dexamethasone eluting stent Italian registry. Heart 2007; 93(5):598-600.

18. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998; 139:4252-4263.

19. Schwartz RS, Chronos NA, Virmani R. Preclinical Restenosis Models and Drug-Eluting Stents. Still Important, Still Much to Learn. J Am Coll Cardiol 2004; 44:1373-1385.

20. Kornowski R, Hong MK, Tio FO, Bramwell O, Wu H, Leon MB. In-Stent Restenosis: Contributions of Inflammatory Responses and Arterial Injury to Neointimal Hyperplasia. J Am Coll Cardiol 1998; 31:224-230.

21. Schwartz RS, Murphy JG, Edwards WD, Camrud AR, Vliestra RE,Holmes DR. Restenosis after balloon angioplasty: a practical proliferative model in porcine coronary arteries. Circulation 1990; 82:2190ï 2200.

22. Schwartz R, Huber K, Murphy J, et al. Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. J Am Coll Cardiol 1992; 19:267ï 274.

23. Schwartz RS, Topol EJ, Serruys PW, Sangiorgi G, Holmes DR Jr. Artery size, neointima, and remodeling: time for some standards. J Am Coll Cardiol 1998; 32:2087ï 2094.

24. Cook S, Walker A, Hügli O, Togni M, Meier B. Percutaneous coronary interventions in Europe: prevalence, numerical estimates, and projections based on data up to 2004. Clin Res Cardiol 2007; 96(6):375-82.

25. Karas SP, Gravanis MB, Santoian EC, Robinson KA, Anderberg KA, King SB III. Coronary intimal proliferation after balloon injury and stenting in swine: an animal model of restenosis. J Am Coll Cardiol 1992; 20:467ï 474.

26. Nakatani M, Takeyama Y, Shibata M, Yorozuya M, Suzuki H, Koba S, Katagiri T. Mechanisms of restenosis after coronary intervention: difference between plain old balloon angioplasty and stenting. Cardiovasc Pathol 2003; 12:40-48.

27. Tanabe K, Regar E, Lee CH, Hoye A, van der Giessen WJ, Serruys W. Local drug delivery using coated stents: new developments and future perspectives. Curr Pharm Des 2004; 10:357-367.

28. Lansky AJ, Costa RA, Mintz GS, Tsuchiya Y, Midei M, Cox DA, et al. Nonpolymer-based paclitaxel-coated coronary stents for the treatment of patients with de novo coronary lesions: angiographic follow-up of the deliver clinical trial. Circulation 2004; 109:1948ï 1954.

29. Kammler J, Hofmann R, Steinwender C, Kypta A, Leisch F. Simultaneous angiographic late stent thrombosis in two different coronary vessels after withdrawal of the combined anti-platelet therapy. Clin Res Cardiol 2006; 95(10):560-4.

30. Scheller B, Speck U, Schmitt A, Bohm M, Nickenig G. Addition of paclitaxel to contrast media prevents restenosis after coronary stent implantation. J Am Coll Cardiol 2003; 42(8):1415-1420.

31. Speck U, Scheller B, Abramjuk C, Grossmann S, Mahnkopf D, Simon O. Inhibition of restenosis in stented porcine coronary arteries: uptake of paclitaxel from angiographic contrast media. Invest Radiol 2004; 39:182-186.

32. Scheller B, Speck U, Abramjuk C, Bernhardt U, Bohm M, Nickenig G. Paclitaxel balloon coating: a novel method for prevention and therapy of restenosis. Circulation 2004; 110:810-814.

33. Speck U, Scheller B, Abramjuk C, Breitwieser C, Dobberstein J, Boehm M, Hamm B. Neointima Inhibition: Comparison of Effectiveness of Nonï Stent-based Local Drug Delivery and a Drug-eluting Stent in Porcine Coronary Arteries. Radiology 2006; 240:411-418.

34. Scheller B, Hehrlein C, Bocksch W, Rutsch W, Haghi D, Dietz U, Böhm M, Speck U. Treatment of in-stent restenosis with a paclitaxel-coated balloon catheter. New Engl J Med 2006; 355:2113-2124.

35. Libby P, Schwartz E, Brogi H, Tanaka H, Clinton SK. A cascade model for restenosis: a special case of atherosclerosis progression. Circulation 1992; 86 Suppl III:47ï 52.

36. Tanaka H, Sukhova GK, Swanson SJ, et al. Sustained activation of vascular cells and leukocytes in the rabbit aorta after balloon injury. Circulation 1993; 88:1788ï 1803.

37. Ribichini F, Joner M, Ferrero V, Finn AV, Crimins J, Nakazawa G, Acampado E, Kolodgie FD, Vassanelli C, Virmani R. Effects of Oral Prednisone After Stenting in a Rabbit Model of Established Atherosclerosis. J Am Coll Cardiol 2007; 50:176-185.

38. Voisard R, Seitzer U, Baur R, et al. Corticosteroid agents inhibit proliferation of smooth muscle cells from human atherosclerotic arteries in vitro. Int J Cardiol 1994; 43:257-267.

39. Nelson SR Chien T, Di Salvo J. Genistein sensitivity of calcium transport pathways in serotonin-activated vascular smooth muscle cells. Arch Biochem Biophys 1997; 345(1):65-72.

40. Si H, Liu D. Phytochemical genistein in the regulation of vascular function: new insights. Curr Med Chem 2007; 14(24):2581-2589.

**Figure legends**

Figure 1. Description of the phases of study analysis (DEB, drug-eluted balloon; SB, standard angioplasty balloon).

Figure 2. Sections perpendicular to the long axis of the vessel, at the position of the mid stent, implanted in a left anterior descending with the Genistein-eluted balloon (A) and in a right coronary artery with a conventional angioplasty balloon (B). In both these four weeks form implantation specimens healing and endothelialization appeared universal. The sections, 100μm thick, were stained with toulidine blue/haematoxylin and eosin/VVG.

Figure 3. Box and whisker plots visualizing median, lower and higher quartiles for the variables analysed at four weeks from implantation, comparing the vessels treated with Genistein-eluted or conventional angioplasty balloon. In order from upper left to lower right neointimal thickness, percentage of lumen stenosis, injury score and mononucleocytes for square millimetre. P values by Studentös *t* test.

Figure 4. Four weeks peri-stent cellularity in a left anterior descending implanted with the conventional angioplasty balloon (focus on total cellularity, A and on mononucleocytes, B) compared to a right coronary artery implanted with the Genistein-eluted balloon (focus on total cellularity, C and on mononucleocytes, D) with the arrows indicating mononucleocytes. The sections, 100μm thick, were stained with toulidine blue/haematoxylin and eosin/VVG.

Figure 5. Box and Whisker plots visualizing median, lower and higher quartiles for the variables analysed at four weeks from implantation, comparing the vessels treated with Genistein-eluted or conventional angioplasty balloon. In order from upper left to lower right neointimal thickness, percentage of lumen stenosis, injury score and mononucleocytes for square millimetre. P values by Studentös *t* test.

**Tables**

Table 1. Histomorphometric and histopathologic measures comparing the ten target vessels treated with Genistein-eluted or conventional angioplasty balloon explanted at four weeks from implantation (o.s., out of stent; i.s., intra-stent; MNCs, mononucleocytes).

| | DEB | N | Mean | Standard deviation | p-value |
|---|---|---|---|---|---|
| **Four weeks post-implantation** | | | | | |
| Equivalent diameter o.s. prox | no | 6 | 1.67 | 0.11 | 0.205 |
| | yes | 4 | 1.42 | 0.43 | |
| Equivalent diameter o.s. dist | no | 6 | 1.59 | 0.20 | 0.915 |
| | yes | 4 | 1.61 | 0.17 | |
| Equivalent diameter i.s. average | no | 6 | 2.64 | 0.32 | 0.450 |
| | yes | 4 | 2.47 | 0.37 | |
| Neointimal thickness average | no | 6 | 0.13 | 0.05 | 0.788 |
| | yes | 4 | 0.14 | 0.06 | |
| % stenosis average | no | 6 | 18.77 | 5.79 | 0.319 |
| | yes | 4 | 22.83 | 6.15 | |
| Injury score average | no | 6 | 0.11 | 0.17 | 0.630 |
| | yes | 4 | 0.18 | 0.32 | |
| Cellularity x mm2 ï total (10^6) | no | 6 | 5715.00 | 731.14 | 0.483 |
| | yes | 4 | 5360.50 | 771.77 | |
| Cellularity x mm2 - MNCs | no | 6 | 95.83 | 28.94 | 0.019 |
| | yes | 4 | 39.00 | 31.91 | |
| Inflammation score | no | 6 | 0.83 | 0.41 | 0.779 |
| | yes | 4 | 0.75 | 0.50 | |

Table 2. Histomorphometric and histopathologic measures comparing the twelve target vessels treated with Genistein-eluted or conventional angioplasty balloon explanted within six and eight weeks from implantation. Abbreviations as in Table 1.

| | DEB | N | Mean | Standard. deviation | p-value |
|---|---|---|---|---|---|
| **Six-eight weeks post-implantation** | | | | | |
| Equivalent diameter o.s. prox | no | 6 | 1.68 | 0.41 | 0.342 |
| | yes | 6 | 1.46 | 0.33 | |
| Equivalent diameter o.s. dist | no | 6 | 1.44 | 0.57 | 0.628 |
| | yes | 6 | 1.59 | 0.47 | |
| Equivalent diameter i.s. average | no | 6 | 2.53 | 0.43 | 0.734 |
| | yes | 6 | 2.61 | 0.37 | |
| Neointimal thickness average | no | 6 | 0.14 | 0.089 | 0.835 |
| | yes | 6 | 0.13 | 0.11 | |
| % stenosis average | no | 6 | 21.66 | 11.23 | 0.534 |
| | yes | 6 | 17.68 | 10.16 | |
| Injury score average | no | 6 | 0.51 | 0.39 | 0.088 |
| | yes | 6 | 0.18 | 0.17 | |
| Cellularity x mm2 ï total (10^6) | no | 6 | 4670.00 | 1197.31 | 0.362 |
| | yes | 6 | 5328.50 | 1192.03 | |
| Cellularity x mm2 - MNCs | no | 6 | 221.50 | 347.20 | 0.307 |
| | yes | 6 | 67.83 | 41.92 | |
| Inflammation score | no | 6 | 1.17 | 0.98 | 0.177 |
| | yes | 6 | 0.50 | 0.55 | |