

Original article

Safety and Efficacy of New Sirolimus-eluting Stent Models in a Preclinical Study



Rodrigo Estévez-Loureiro,^{a,*} Armando Pérez de Prado,^a Claudia Pérez-Martínez,^a Carlos Cuellas-Ramón,^a Marta Regueiro-Purriños,^a José M. Gonzalo-Orden,^a María López-Benito,^a María Molina-Crisol,^b Luis Duocastella-Codina,^b and Felipe Fernández-Vázquez^a

^a Grupo Cardiovascular (HemoLeon), Fundación Investigación Sanitaria en León y del Instituto de Biomedicina (IBIOMED), Universidad de León, Hospital de León, León, Spain

^b Life Vascular Devices (LVD) Biotech, iVascular, Barcelona, Spain

Article history:

Received 17 December 2014

Accepted 18 February 2015

Available online 21 July 2015

Keywords:

Preclinical model
Drug-eluting stent
Restenosis
Vascular healing

ABSTRACT

Introduction and objectives: Initial preclinical studies are required during the process of improving polymers, platforms, and drug-eluting systems for new coronary stent designs. Our objective was to analyze the efficacy and safety of new drug-eluting stent models compared with a conventional stent and commercialized drug-eluting stents in an experimental model with healthy porcine coronary arteries.

Methods: Sixty stents (conventional stent, new sirolimus-eluting stents: drug-eluting stents 1, 2 and 3; Cypher[®] and Xience[®]) were randomly placed in the coronary arteries of 20 Large White domestic pigs. Angiographic and histomorphometric studies were done 28 days later.

Results: The stents were implanted at a stent/artery ratio of 1.34 ± 0.15 , with no significant differences between groups. The new stents showed less late loss and angiographic restenosis than conventional stents ($P = .006$ and $P < .001$, respectively). Histologically, restenosis and neointimal area were lower with all the new platforms than with the conventional stents ($P < .001$ for each variable), and no differences were found vs the drug-eluting stents on the market. Safety data showed that endothelialization was lower with drug-eluting stents than with conventional stents, except for drug-eluting stent 3 ($P = .084$). Likewise, inflammation was lower with drug-eluting stent 3 than with other stents.

Conclusions: The new drug-eluting stent platforms studied are associated with less restenosis than conventional stents and showed no significant differences in safety or efficacy vs commercialized drug-eluting stents.

© 2015 Sociedad Española de Cardiología. Published by Elsevier España, S.L.U. All rights reserved.

Seguridad y eficacia de nuevos modelos de stents liberadores de sirolimus en el modelo preclínico

RESUMEN

Introducción y objetivos: En el proceso de mejora de los polímeros, las plataformas y los sistemas de liberación de fármacos en los nuevos diseños de stents farmacoactivos, el análisis preclínico inicial es obligatorio. El objetivo es analizar la eficacia y la seguridad de nuevos modelos de stents farmacoactivos en comparación con un stent convencional y stents farmacoactivos comercializados en el modelo experimental de arteria coronaria sana porcina.

Métodos: Se implantaron aleatoriamente 60 stents (stent convencional, nuevos stents liberadores de sirolimus: stents liberadores de fármaco 1, 2 y 3; Cypher[®] y Xience[®]) en las arterias coronarias de 20 cerdos domésticos raza Large White. Se realizó estudio angiográfico e histomorfométrico a los 28 días.

Resultados: Los stents se implantaron en proporción stent/arteria de $1,34 \pm 0,15$, sin diferencias significativas entre grupos. Los nuevos stents mostraron menos pérdida tardía y restenosis angiográfica que los convencionales ($p = 0,006$ y $p < 0,001$ respectivamente). Todas las nuevas plataformas presentaron menos área neointimal y restenosis histológica que los stents convencionales ($p < 0,001$ para cada variable), sin diferencias con los farmacoactivos comercializados. En cuanto a la seguridad, todos los stents farmacoactivos mostraron menos endotelización que los convencionales, salvo el stent liberador de fármaco 3 ($p = 0,084$). Asimismo, la inflamación observada fue menor con el stent liberador de fármaco 3 que con los demás.

Palabras clave:

Modelo preclínico
Stent liberador de fármaco
Restenosis
Reparación vascular

* Corresponding author: Cardiología Intervencionista, Hospital de León, Altos de Nava s/n, 24008 León, Spain.
E-mail address: roiestevez@hotmail.com (R. Estévez-Loureiro).

Conclusiones: Las nuevas plataformas de *stents* farmacoactivos estudiadas se asocian con menos restenosis que los convencionales, sin diferencias significativas en seguridad y eficacia respecto a los *stents* farmacoactivos comercializados.

© 2015 Sociedad Española de Cardiología. Publicado por Elsevier España, S.L.U. Todos los derechos reservados.

Abbreviations

CS: conventional stent
DES: drug-eluting stent
MLD: minimal luminal diameter

INTRODUCTION

Drug-eluting stents (DES) are one of the greatest advances made in the percutaneous treatment of coronary disease. These devices have consistently provided lower rates of target vessel revascularization than conventional stents (CS) in a wide range of clinical situations.^{1–4} However, the risk of late and very late thrombosis associated with these stents is still a cause for concern.^{5,6} This phenomenon has been related to the deleterious effects of the drug, polymer, stent platform, or a combination of all 3 on the vessel wall, leading to incomplete endothelialization, persistent inflammatory reactions, and the development of neoatherosclerosis.^{7–11}

To overcome these limitations, innovations have been made in platform design and drug-eluting systems, polymers have been developed that are more biocompatible or resorbable, and even a completely resorbable DES has been designed.^{12–14} Preclinical animal studies have been deemed very useful for analyzing the differences among new devices because the sequence of biological events associated with arterial healing (injury caused by the stent, fibrin deposits, inflammation and cell proliferation) is similar to that in humans.^{15–17} Experimental models with healthy porcine coronary arteries are considered suitable for evaluating biological responses after the placement of CS, DES or drug-eluting balloons.^{18–20}

The purpose of this study was to evaluate the safety and efficacy of 3 new sirolimus-eluting permanent polymer stent designs in terms of vascular response in a preclinical porcine model.

METHODS

Animal Model

In this randomized, controlled, experimental study with a final blind analysis, we used 20 Large White domestic pigs aged 2 to 3 months old and weighing 25 ± 3 kg. All procedures were done in accordance with local regulations (RD 53/2013, February 1, which defines basic standards for the protection of animals during experimentation and other scientific purposes, such as education) and European Directive 2010/63/EC. Before any procedures were initiated, the study was approved by the local ethics committee.

The randomization method involved the stratified allocation of major coronary arteries in such a way that each stent type was implanted in the same number of arteries.

All animals received antiplatelet therapy with acetylsalicylic acid (325 mg) and clopidogrel (300 mg) 24 hours before the procedure. The anesthesia protocol and surgical preparation have been previously described.^{21,22} The animals were anesthetized and received anticoagulant therapy with 5000 IU of unfractionated

heparin. Coronary angiography was performed via left carotid artery access after intracoronary administration of nitroglycerin.

Angioplasty Procedure

To implant the devices and obtain a stent/artery ratio > 1.1 , we selected the best location out of the 3 epicardial coronary arteries. After inserting an intracoronary guidewire, the different stent types were implanted in the selected area of each artery.

Devices Analyzed

For this study, we used the following devices (numbers in parentheses):

1. Control CS (n = 11): L605 cobalt chromium alloy stent, Architect[®] (iVascular). The stent is constructed of 6 crowns joined by 3 rows of concatenated connectors that create a continuous sinusoidal structure (Figure 1A).
2. DES 1 (n = 17): based on the metal Architect[®] stent, coated with a permanent polyacrylate polymer and loaded with $1.4 \mu\text{g}/\text{mm}^2$ of sirolimus in a slow-release system (with an additional polymeric external layer to control drug release).
3. DES 2 (n = 10): based on the metal Architect[®] stent, coated with a permanent polyacrylate polymer and loaded with $1.4 \mu\text{g}/\text{mm}^2$ of sirolimus (and no external polymer barrier).
4. DES 3 (n = 12): is the new DES by iVascular, called Angiolite[®]. The platform is made of L605 cobalt chromium alloy, with a strut thickness of $85 \mu\text{m}$. The stent structure has 8 crowns joined by 3 rows of unlinked connectors that create a discontinuous sinusoidal structure (Figure 1B). This design represents a slight increase in the metal/artery ratio and provides better distribution of the drug to the artery wall. The polymer is permanent and from the polyacrylate family. It releases sirolimus at a dose of $1.4 \mu\text{g}/\text{mm}^2$, and more than 80% of the drug is released within 60 days.

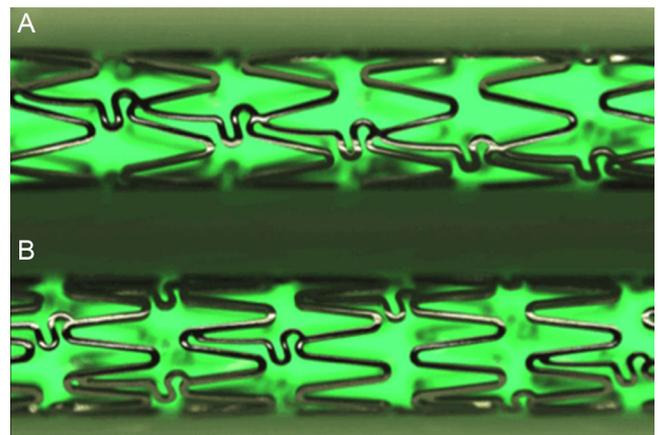


Figure 1. Stent design of the conventional cobalt chromium Architect[®], the metallic control platform, drug-eluting stent 1 and drug-eluting stent 2 (A); the new metallic structural platform of the drug-eluting stent 3 has more crowns per segment and unlinked connectors to provide more uniform elution (B). High definition images using the QSix[®] system (Barcelona, Spain).

5. DES 4 (n = 5): commercial first-generation Cypher® DES (J&J Cordis; Miami Lakes, Florida, United States) with a strut thickness of 140 μm , loaded with 1.4 $\mu\text{g}/\text{mm}^2$ of sirolimus, and permanent copolymer matrix.
6. DES 5 (n = 5): second-generation Xience® DES (Abbott Vascular; Santa Clara, California, United States). This stent elutes everolimus at a dose of 1 $\mu\text{g}/\text{mm}^2$ through its permanent fluorinated copolymer. The metallic platform is a Multi-Link stent, composed of a cobalt chromium alloy, with a strut thickness of 81 μm .

All the material was provided by iVascular, including DES 1, DES 2 and DES 3, which are still not commercially available.

The sample size and number of stents included in the study were calculated according to consensus documents on preclinical stent analysis.²⁰

Angiographic Studies

After the above-mentioned procedures were completed in each artery, coronary catheterization was repeated (after intracoronary administration of nitroglycerin) to determine the minimal luminal diameter (MLD) inside each stent. Catheterization was repeated 28 days later to evaluate follow-up MLD. Both MLDs, after the procedure and during follow-up, as well as the reference diameters of the arteries (mean diameter of the arterial segments located in the proximal and distal 5 mm of the stent), were calculated with Medis QCA-CMS (version 6.1) software for quantitative coronary analysis. The following angiographic restenosis parameters were calculated:

- Late loss = initial MLD – follow-up MLD.
- Percentage of angiographic restenosis = $[1 - (\text{initial MLD} / \text{reference diameter})] \times 100$.

The presence of overlapping branches that impeded correct vessel measurement was considered a criterion for exclusion from the angiographic analysis.

Histologic Analysis

After completing the angiographic follow-up, the animals were killed and underwent complete histologic analysis. The hearts were explanted and coronary arteries were preserved by means of pressure perfusion, which was done initially with phosphate buffered saline and later using 4% paraformaldehyde. The treated segments were dissected, and the 5 mm distal and proximal to the treated area were preserved. The samples were embedded in plastic resins to obtain circumferential sections representative of the proximal, medial, and distal areas and to calculate the mean values of each segment studied. Afterward, the sections were systematically stained with hematoxylin-eosin and Van Gieson stain.

The arteries were analyzed histomorphometrically with an Olympus PRovis AX70W digital microscope (Tokyo, Japan) paired with a Nikon DXM 1200W digital camera and ImageJ-NIH Image 1.4 software (National Institutes of Health, United States). Planimetry was used to determine the luminal area and the internal elastic lamina area and thus calculate restenosis variables defined by histology:

- Neointimal area = area of internal elastic – luminal area.
- Percentage of stenosis by histology = $[1 - (\text{luminal area} / \text{internal elastic area})] \times 100$.

The histologic analysis in terms of safety was based on semiquantitative analysis of 4 parameters: degree of vascular

injury (injury score), defined by Schwartz et al²³; inflammation intensity, defined by Kornowski et al²⁴; persistent fibrin deposition, according to Suzuki et al.²⁵ and the degree of re-endothelialization, calculated as the approximate percentage of luminal surface covered by endothelial cells. According to the amount of surface covered by endothelial cells 28 days after implantation, an additional parameter was defined: complete endothelialization, which was at least 95% of the luminal surface covered by the endothelial cells.²⁶ Stents with an injury score > 2 were excluded from the final analysis as they can have nonspecific responses in the histology of the arterial wall.

Statistical Method

Values are presented as proportions and as mean \pm standard deviation, depending on the type of variable. The semiquantitative variables, like the safety scores in the histopathologic analysis, are described as mean \pm standard deviation (the most common form in previous publications) and as percentages (recommended by consensus documents for preclinical stent studies).^{20,27}

We analyzed the differences between the mean of the groups using Student's *t* test and analysis of variance. For multiple comparisons, a *post-hoc* analysis was done with the Dunnett method for comparison with the control CS and with the Tukey method for comparison of all groups. The semiquantitative variables were analyzed with the chi-square test or Fisher's exact method. To evaluate the possible influence of different variables (stent/artery ratio, treated artery, stent type and injury score) on the final results of angiographic and histologic stenosis, we carried out a multivariate linear regression analysis that included, in addition to the cited variables, the stent type (CS or DES). The variables were entered into the model as a block with a *P* value for entry of .05 and an output *P* of .1. For the analyses, a *P* < .05 was considered significant.

RESULTS

In this study, we finally implanted 59 of the 60 planned stents in porcine coronary arteries (1 CS could not be correctly implanted in the right coronary artery) and overdilated to a mean stent/artery ratio of 1.34 ± 0.15 , with no significant differences between stents. The animals completed the planned follow-up; angiographic follow-up studies and histologic analyses were done without incident. All the treated segments were permeable in the final angiographic analysis. Two CS, 2 DES-1 and 1 DES-3 were excluded from the final analysis because they had an injury score > 2. The angiographic analysis of 2 stents could not be completed due to the presence of overlapping branches in the stent segment of 1 case (DES-1 in circumflex artery) and inadequate opacification of the stent in the other (DES-3 in right coronary).

Analysis of Antirestenotic Effectiveness

Antirestenotic properties are shown in Tables 1 and 2. In general, late loss was significantly lower for all the DES than for the control CS (*P* = .006). Late loss was lower for DES-1 and DES-3 than for the CS (*P* = .025 and *P* = .004, respectively). Likewise, values for this variable were lower for DES-3 than for the Xience® stent, a finding that was borderline statistically significant (*P* = .049). Angiographic restenosis was also lower in the DES group than in the control CS (*P* < .001); nevertheless, this was only true with the new prototypes tested (*P* < .001 for DES-1; *P* = .047 for DES-2; *P* < .001 for DES-3). Restenosis was lower for DES-3 than for the Xience® stent (*P* = .011) (Figure 2).

Table 1
Angiographic Results at 28 Days

	CS (n=8)	DES-1 (n=14)	DES-2 (n=10)	DES-3 (n=11)	Xience® (n=5)	Cypher® (n=5)	P (CS vs DES)
Late loss, mm	1.54 ± 0.40	1.03 ± 0.55	1.26 ± 0.30	0.87 ± 0.30	1.52 ± 0.37	1.19 ± 0.30	.006
Stenosis, %	37.11 ± 12.73	15.89 ± 10.59	22.89 ± 7.92	9.73 ± 6.09	28.96 ± 9.86	22.74 ± 14.14	< .001

CS, conventional stent; DES, drug-eluting stent.
Data are shown as mean ± standard deviation.

Table 2
Histology Results at 28 Days

	CS (n=8)	DES-1 (n=15)	DES-2 (n=10)	DES-3 (n=12)	Xience® (n=5)	Cypher® (n=5)	P (CS vs DES)
Neointimal area, mm ²	3.70 ± 0.91	2.20 ± 0.74	2.43 ± 0.65	1.84 ± 0.60	1.93 ± 0.84	3.35 ± 0.88	< .001
Area of stenosis, %	63.55 ± 15.39	36.31 ± 14.17	41.02 ± 10.83	35.17 ± 11.04	29.60 ± 9.58	46.92 ± 8.19	< .001
Injury score	0.96 ± 0.44	0.89 ± 0.55	0.87 ± 0.42	0.56 ± 0.27	0.87 ± 0.47	0.81 ± 0.48	.318
Injury score ≥ 1, %	38	40	30	0	40	40	.249
Inflammation score	0.99 ± 0.63	0.86 ± 0.44	0.89 ± 0.46	0.29 ± 0.12	0.80 ± 0.38	1.16 ± 0.46	.198
Inflammation score ≥ 1, %	25	40	40	0	20	60	.111
Fibrin score	0.33 ± 0.40	1.36 ± 0.51	1.25 ± 0.54	1.41 ± 0.47	1.40 ± 0.43	1.67 ± 0.53	<.001
Fibrin score ≥ 1, %	25	40	40	0	20	60	.249
Endothelialized surface, %	99.63 ± 1.10	94.22 ± 3.18	96.0 ± 1.10	96.75 ± 1.26	94.40 ± 2.60	95.40 ± 3.65	<.001
Complete endothelialization (> 95%), %	100	47	50	83	20	40	.005

CS, conventional stent; DES, drug-eluting stent.
Unless otherwise indicated, the data are shown as mean ± standard deviation.

The histologic analysis showed that neointimal area and histologic restenosis were lower with the new sirolimus DES than with the CS ($P < .001$ for both variables). Neointimal area was significantly lower with the new devices than with the CS and the Cypher®, but no differences were found vs the Xience® stent. Likewise, histologic restenosis was lower with the new devices than with the CS, but was higher than with Xience® or Cypher®. The effects of the different stents, both in the angiography and in the histology studies are shown in Figure 3.

A multivariate analysis evaluated the influence of different variables on histologic restenosis. An independent association was found between a greater degree of restenosis and a higher injury score ($B = 13.38$; 95% confidence interval, 5.68–21.08; $P < .001$) and lower levels in the case of DES vs control ($B = -26.73$; 95% confidence interval, -35.95 to 17.51; $P < .001$).

Safety Analysis

Safety variables are shown in Table 2. In general, at 28 days, endothelialization was greater with the CS than with the analyzed

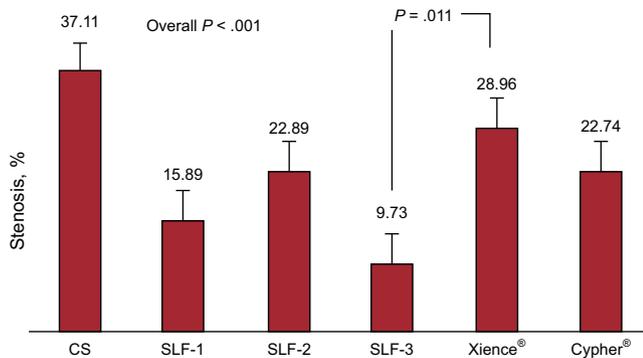


Figure 2. Angiographic restenosis was significantly higher in conventional stent than in drug-eluting stent. CS, conventional stent; DES, drug-eluting stent.

DES ($P < .001$). This difference was not statistically significant in the case of DES-3 ($P = .084$). No differences were observed among the DES analyzed. The injury score showed no significant differences between the devices. The degree of inflammation was even lower for DES-3 than for the CS ($P = .010$) (Figure 4). Lastly, fibrin deposits were lower in the CS group than in the tested DES ($P < .001$).

DISCUSSION

This study analyzed the safety and efficacy of new sirolimus-eluting stent models with a permanent polymer compared with CS and 2 currently available DES. Our preclinical model showed that the new devices reduce the degree of restenosis (fundamentally DES-1 and DES-3) vs the control CS, with similar safety parameters as currently available DES.

Preclinical animal models are a fundamental part of the approval process and are essential for verifying the safety and efficacy of new coronary devices. Likewise, these models are the most effective method to evaluate mural response to new therapeutic materials.

The new DES models generally demonstrated greater efficacy in the prevention of restenosis than the control CS. This finding was expected, as previous studies had reported that different sirolimus-eluting stents significantly reduce late loss and target vessel revascularization compared with CS.^{1,2,25,28–30} Furthermore, in our study, DES-3 seemed to be the most effective stents in general, even when compared with commercially-available DES. This finding is relevant because recent meta-analyses have reported that the Xience® stent is the device with the best efficacy/safety ratio.^{31,32} Although this finding may be explained by various factors, previous publications indicate that neointimal hyperplasia and thrombus deposition can be significantly affected by stent design and coating.^{33–36} It is likely that these effects may be caused by the new design of this platform, with improved distribution of the drug along the artery wall. Despite these promising results, we must assume that the porcine coronary

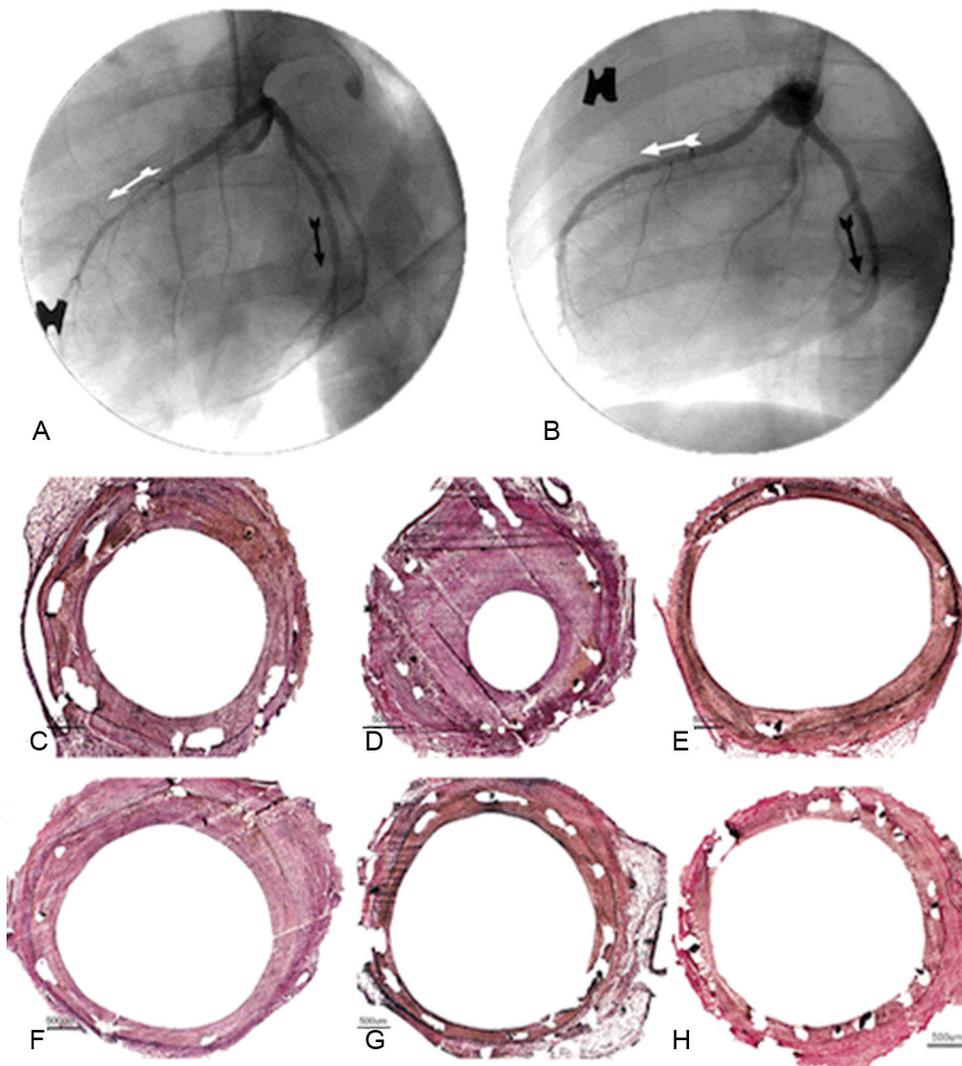


Figure 3. Antiproliferative effectiveness: angiographic and histologic comparison at 28 days. Angiographic results of conventional control stent (A: anterior descending, white arrow), drug-eluting stent 3 (A: circumflex, black arrow), drug-eluting stent 2 (B: anterior descending, white arrow) and drug-eluting stent 1 (B: circumflex, black arrow). Histology results of the Cypher[®] stent (C), conventional stent (D), Xience[®] (E), drug-eluting stent 1 (F), drug-eluting stent 2 (G) and drug-eluting stent 3 (H).

model has limitations for assessing mural response to stents, and that no animal model completely and precisely reproduces the characteristics of vascular disease in humans.^{20,27} This positive finding should therefore be interpreted with caution. Nonetheless, experimental studies indicate that there are important similarities

between the inflammation, vascular injury and neointimal growth of these models and native human coronary arteries, although the vascular injury in animal models differs from that of atherosclerotic human arteries.^{37,38} Vascular injury is the fundamental event that could lead to the differences found among the stents studied in the porcine model. Our group has previously reported that a greater degree of vascular injury caused by stents over-dilatation is a powerful stimulus for neointimal proliferation. Therefore, the greater the vascular damage, the greater the efficacy of the DES in preventing neointimal response.²² This phenomenon was found in our study, in which the stent/artery ratio was higher than recommended,²⁰ which allowed differences to be observed among the devices. Furthermore, it is relevant that this over-dilatation was not associated with an excessive injury score, which could have altered the results of the study by inducing nonspecific vascular responses. This would be confirmed by the absence of a significant association between this variable and the degree of angiographic or histologic stenosis. In our multivariate analysis, the use of DES was independently associated with a lower degree of stenosis, confirming that the effect is real and is not related to arterial injury.

The safety data showed that the degree of endothelialization was lower in the DES than in the control CS, which can be explained by the effect of sirolimus, a powerful inhibitor of endothelial

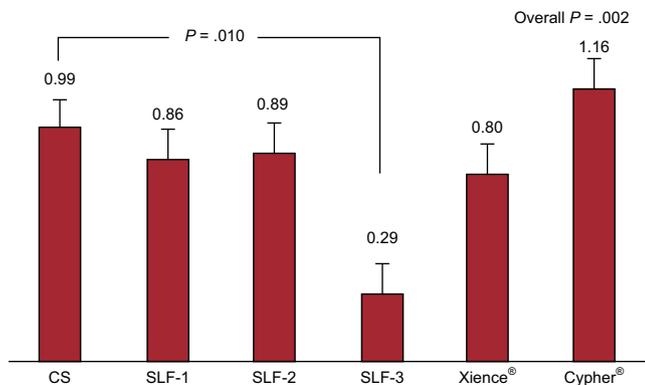


Figure 4. Inflammation score: the lower levels with the drug-eluting stent 3 stent are of interest. CS, conventional stent; DES, drug-eluting stent.

proliferation that deactivates the p70 S6 kinase pathway, an essential step for the progression of the cell cycle in response to growth factors.^{28,39,40} Interestingly, DES-3 showed no significant differences compared with CS, confirming the safety of this device. Likewise, fibrin deposition around the stents was greater in the DES group, which is an indication of the effect of the drug.⁴¹ Importantly, injury scores were low for all the stents, with a mean < 1 despite a stent/artery ratio \geq 1.3. This finding is probably a consequence of the high degree of biocompatibility of the tested material. Lastly, inflammatory response was lower with DES-3 than with the control CS. Although this finding could be explained by several factors, it is possible that this new design, with its improved sirolimus-eluting pattern, may be associated with a greater anti-inflammatory effect of the drug,⁴² which could counterbalance the proinflammatory effect of the permanent polymer.⁴³

Limitations

Similar to all preclinical models, this study has inherent limitations because no animal model can exactly reproduce the complex characteristics of human coronary disease. Animal models of disease can replicate some of them, but their exact interpretation is not quite clear. Although the proliferative response obtained is able to evaluate drug activity and stent behavior, it is unknown whether this effect would be the same in arteries with a large atherosclerotic content, in which fragmentation of the internal elastic lamina spontaneously appears as part of the general inflammatory process, especially in vulnerable plaques, in which other mediators participate in the vascular healing process. Furthermore, patients with atherosclerosis have other molecules and genetic factors that can directly interfere in the process. Nevertheless, the porcine coronary artery model continues to be recommended by consensus documents for the evaluation of these devices. Accuracy is also limited by histologic evaluation using semiquantitative variables; nonetheless, we have followed the standards for analysis postulated by expert consensus.^{20,27} The sample size was calculated in accordance with the standards defined by consensus documents for preclinical studies of coronary stents. Even though the power for detecting differences with CS is adequate, the power to detect differences among DES types is lower. These comparisons should therefore be interpreted with caution.

CONCLUSIONS

In our experimental model with healthy porcine coronary arteries implanted with oversized stents, the new sirolimus stents tested significantly reduced restenosis compared with the control CS. In addition, we found no relevant differences with the first- and second-generation DES currently available on the market.

FUNDING

This study was completed with the support of the CDTI (Center for Industrial Technological Development) of the Spanish Ministry of Economics and Competitiveness (IDI-20111025).

CONFLICTS OF INTEREST

A. Pérez de Prado and F. Fernández-Vázquez are consultants and receive funding for different research projects from LVD Biotech/iVascular. M. Molina and L. Duocastella are employed by LVD

Biotech/iVascular. L. Duocastella is the CEO (Chief Executive Officer) and holds shares in LVD Biotech/iVascular.

REFERENCES

- Spaulding C, Daemen J, Boersma E, Cutlip DE, Serruys PW. A pooled analysis of data comparing sirolimus-eluting stents with bare-metal stents. *N Engl J Med*. 2007;356:989–97.
- Kastrati A, Mehilli J, Pache J, Kaiser C, Valgimigli M, Kelbaek H, et al. Analysis of 14 trials comparing sirolimus-eluting stents with bare-metal stents. *N Engl J Med*. 2007;356:1030–9.
- Stone GW, Moses JW, Ellis SG, Schofer J, Dawkins KD, Morice MC, et al. Safety and efficacy of sirolimus- and paclitaxel-eluting coronary stents. *N Engl J Med*. 2007;356:998–1008.
- Sarno G, Lagerqvist B, Frøbert O, Nilsson J, Olivecrona G, Ömerovic E, et al. Lower risk of stent thrombosis and restenosis with unrestricted use of 'new-generation' drug-eluting stents: a report from the nationwide Swedish Coronary Angiography and Angioplasty Registry (SCAAR). *Eur Heart J*. 2012;33:606–13.
- Daemen J, Wenaweser P, Tsuchida K, Abrecht L, Vaina S, Morger C, et al. Early and late coronary stent thrombosis of sirolimus-eluting and paclitaxel-eluting stents in routine clinical practice: data from a large two-institutional cohort study. *Lancet*. 2007;369:667–78.
- Stettler C, Wandel S, Allemann S, Kastrati A, Morice MC, Schömig A, et al. Outcomes associated with drug-eluting and bare-metal stents: a collaborative network meta-analysis. *Lancet*. 2007;370:937–48.
- Finn AV, Joner M, Nakazawa G, Kolodgie F, Newell J, John MC, et al. Pathological correlates of late drug-eluting stent thrombosis: strut coverage as a marker of endothelialization. *Circulation*. 2007;115:2435–41.
- Nakazawa G, Finn AV, Vorpahl M, Ladich ER, Kolodgie FD, Virmani R. Coronary responses and differential mechanisms of late stent thrombosis attributed to first-generation sirolimus- and paclitaxel-eluting stents. *J Am Coll Cardiol*. 2011;57:390–8.
- Nakazawa G, Otsuka F, Nakano M, Vorpahl M, Yazdani SK, Ladich E, et al. The pathology of neoatherosclerosis in human coronary implants bare-metal and drug-eluting stents. *J Am Coll Cardiol*. 2011;57:1314–22.
- Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, et al. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. *J Am Coll Cardiol*. 2006;48:193–202.
- Virmani R, Guagliumi G, Farb A, Musumeci G, Grieco N, Motta T, et al. Localized hypersensitivity and late coronary thrombosis secondary to a sirolimus-eluting stent: should we be cautious? *Circulation*. 2004;109:701–5.
- Abizaid A, Costa Jr JR. New drug-eluting stents: an overview on biodegradable and polymer-free next-generation stent systems. *Circ Cardiovasc Interv*. 2010;3:384–93.
- Serruys PW, Ormiston JA, Onuma Y, Regar E, Gonzalo N, Garcia-Garcia HM, et al. A bioabsorbable everolimus-eluting coronary stent system (ABSORB): 2-year outcomes and results from multiple imaging methods. *Lancet*. 2009;373:897–910.
- Serruys PW, Onuma Y, Dudek D, Smits PC, Koolen J, Chevalier B, et al. Evaluation of the second generation of a bioresorbable everolimus-eluting vascular scaffold for the treatment of de novo coronary artery stenosis: 12-month clinical and imaging outcomes. *J Am Coll Cardiol*. 2011;58:1578–88.
- Farb A, Sangiorgi G, Carter AJ, Walley VM, Edwards WD, Schwartz RS, et al. Pathology of acute and chronic coronary stenting in humans. *Circulation*. 1999;99:44–52.
- Nakazawa G, Ladich E, Finn AV, Virmani R. Pathophysiology of vascular healing and stent mediated arterial injury. *EuroIntervention*. 2008;4 Suppl C:7–10.
- Schwartz RS, Chronos NA, Virmani R. Preclinical restenosis models and drug-eluting stents: still important, still much to learn. *J Am Coll Cardiol*. 2004;44:1373–85.
- Pérez de Prado A, Pérez Martínez C, Cuellas Ramon C, Gonzalo Orden JM, Altonaga JR, Iglesias MJ, et al. Endothelialization of nonapposed stent struts located over the origin of a side branch: results with different carboxymethylated stents. *J Interv Cardiol*. 2009;22:222–7.
- Joner M, Byrne RA, Lapointe JM, Radke PW, Bayer G, Steigerwald K, et al. Comparative assessment of drug-eluting balloons in an advanced porcine model of coronary restenosis. *Thromb Haemostasis*. 2011;105:864–72.
- Schwartz RS, Edelman E, Virmani R, Carter A, Granada JF, Kaluza GL, et al. Drug-eluting stents in preclinical studies: updated consensus recommendations for preclinical evaluation. *Circ Cardiovasc Interv*. 2008;1:143–53.
- Pérez de Prado A, Pérez-Martínez C, Cuellas-Ramón C, Gonzalo-Orden JM, Regueiro-Purriños M, Martínez B, et al. Time course of reendothelialization of stents in a normal coronary swine model: characterization and quantification. *Vet Pathol*. 2011;48:1109–17.
- Diego A, Pérez de Prado A, Cuellas C, Pérez-Martínez C, Gonzalo-Orden M, Altonaga JR, et al. La restenosis en el stents depende del daño vascular inducido ¿Son válidos los modelos experimentales actuales de análisis de los stents farmacoactivos? *Rev Esp Cardiol*. 2011;64:745–51.
- Schwartz RS, Huber KC, Murphy JG, Edwards WD, Camrud AR, Vlietstra RE, et al. Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. *J Am Coll Cardiol*. 1992;19:267–74.
- 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;31:224–30.

25. Suzuki T, Kopia G, Hayashi S, Bailey LR, Llanos G, Wilensky R, et al. Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. *Circulation*. 2001;104:1188–93.
26. Perez de Prado A, Perez-Martinez C, Cuellas C, Gonzalo-Orden JM, Diego A, Regueiro M, et al. Preclinical evaluation of coronary stents: focus on safety issues. *Curr Vasc Pharmacol*. 2013;11:74–99.
27. Schwartz RS, Edelman ER, Carter A, Chronos N, Rogers C, Robinson KA, et al. Drug-eluting stents in preclinical studies: recommended evaluation from a consensus group. *Circulation*. 2002;106:1867–73.
28. Joner M, Nakazawa G, Finn AV, Quee SC, Coleman L, Acampado E, et al. Endothelial cell recovery between comparator polymer-based drug-eluting stents. *J Am Coll Cardiol*. 2008;52:333–42.
29. Spaulding C, Henry P, Teiger E, Beatt K, Bramucci E, Carrié D, et al. Sirolimus-eluting versus uncoated stents in acute myocardial infarction. *N Engl J Med*. 2006;355:1093–104.
30. Spaulding C, Teiger E, Commeau P, Varenne O, Bramucci E, Slama M, et al. Four-year follow-up of TYPHOON (trial to assess the use of the CYPHer sirolimus-eluting coronary stent in acute myocardial infarction treated with Balloon angioplasty). *JACC Cardiovasc Interv*. 2011;4:14–23.
31. Palmerini T, Biondi-Zoccai G, Della Riva D, Mariani A, Sabaté M, Valgimigli M, et al. Clinical outcomes with drug-eluting and bare-metal stents in patients with ST-segment elevation myocardial infarction: evidence from a comprehensive network meta-analysis. *J Am Coll Cardiol*. 2013;62:496–504.
32. Palmerini T, Biondi-Zoccai G, Della Riva D, Mariani A, Sabaté M, Smits PC, et al. Clinical outcomes with bioabsorbable polymer- versus durable polymer-based drug-eluting and bare-metal stents: evidence from a comprehensive network meta-analysis. *J Am Coll Cardiol*. 2014;63:299–307.
33. Garasic JM, Edelman ER, Squire JC, Seifert P, Williams MS, Rogers C. Stent and artery geometry determine intimal thickening independent of arterial injury. *Circulation*. 2000;101:812–8.
34. Rogers C, Edelman ER. Endovascular stent design dictates experimental restenosis and thrombosis. *Circulation*. 1995;91:2995–3001.
35. Kolandaivelu K, Swaminathan R, Gibson WJ, Kolachalama VB, Nguyen-Ehrenreich KL, Giddings VL, et al. Stent thrombogenicity early in high-risk interventional settings is driven by stent design and deployment and protected by polymer-drug coatings. *Circulation*. 2011;123:1400–9.
36. Balakrishnan B, Tzafriri AR, Seifert P, Groothuis A, Rogers C, Edelman ER, et al. Strut position, blood flow, and drug deposition: implications for single and overlapping drug-eluting stents. *Circulation*. 2005;111:2958–65.
37. Schwartz RS, Henry TD. Pathophysiology of coronary artery restenosis. *Rev Cardiovasc Med*. 2002;3 Suppl 5:4–9.
38. Farhan S, Hemetsberger R, Matiassek J, Strehblow C, Pavo N, Khorsand A, et al. Implantation of paclitaxel-eluting stent impairs the vascular compliance of arteries in porcine coronary stenting model. *Atherosclerosis*. 2009;202:144–51.
39. Steigerwald K, Ballke S, Quee SC, Byrne RA, Vorpahl M, Vogeser M, et al. Vascular healing in drug-eluting stents: differential drug-associated response of limus-eluting stents in a preclinical model of stent implantation. *EuroIntervention*. 2012;8:752–9.
40. Finn AV, Nakazawa G, Joner M, Kolodgie FD, Mont EK, Gold HK, et al. Vascular responses to drug eluting stents: importance of delayed healing. *Arterioscler Thromb Vasc Biol*. 2007;27:1500–10.
41. Pérez A, Pérez-Martínez C, Ramón C, Regueiro Purriños M, Diego Nieto A, Gonzalo-Orden JM, et al. Análisis de la eficacia y la seguridad de distintos balones liberadores de paclitaxel en un modelo animal. *Rev Esp Cardiol*. 2014;67:456–62.
42. Sehgal SN. Rapamune (sirolimus, rapamycin): an overview and mechanism of action. *Ther Drug Monit*. 1995;17:660–5.
43. Yazdani SK, Sheehy A, Nakano M, Nakazawa G, Vorpahl M, Otsuka F, et al. Preclinical evaluation of second-generation everolimus- and zotarolimus-eluting coronary stents. *J Invasive Cardiol*. 2013;25:383–90.