https://doi.org/10.17221/159/2019-VETMED

Adventitial histopathological changes after coronary stenting in a porcine model

Claudia Perez-Martinez[1,2], Armando Perez de Prado[2,3]*, Vanesa Caballero-Manso[1], Marta Regueiro-Purrinos[2,4], Mª Gracia de Garnica Garcia[5], Carlos Cuellas-Ramon[2,3], Jose Manuel Gonzalo-Orden[2,4], Maria Lopez-Benito[2,3], Jose R. Altonaga[2,4], Tomas Benito-Gomez[2,3], Felipe Fernandez-Vazquez[2,3]*

[1]*Department of Animal Health, Section of Pathology, Veterinary School, University of León, León, Spain*
[2]*Institute of Biomedicine (IBIOMED), University of León, León, Spain*
[3]*Health Research Foundation in León, León, Spain*
[4]*Department of Veterinary Medicine, Surgery and Anatomy, Section of Surgery, Veterinary School, University of León, León, Spain*
[5]*MicrosVeterinaria SL, León, Spain*

**Corresponding author: cperm@unileon.es*

Claudia Perez-Martinez and Armando Perez de Prado contributed equally to this work

Citation: Perez-Martinez C, Perez de Prado A, Caballero-Manso V, Regueiro-Purrinos M, de Garnica Garcia MG, Cuellas-Ramon C, Gonzalo-Orden JM, Lopez-Benito M, Altonaga JR, Benito-Gomez T, Fernandez-Vazquez F (2020): Adventitial histopathological changes after coronary stenting in a porcine model. Vet Med-Czech 65, 465–472.

Abstract: The adverse long-term events in first-generation drug-eluting stents were associated with chronic inflammatory response to the polymer. As an alternative, stents with biodegradable polymers emerged, whose effects on the vascular response are not yet fully known. Our objectives were to study the adventitial response to the stent implantation and the role of the polymeric vehicle. A histological (Haematoxylin-Eosin, Verhoeff van Gieson) and immunohistochemical (von Willebrand factor, alpha-smooth muscle actin) analysis were performed on resin-embedded arterial sections from fifteen Large White pigs, 28 days after the random implantation in the coronary arteries of: a chromium-cobalt stent and a stent coated with a permanent polyacrylate or biodegradable poly(D,L)-lactic-co-glycolic polymer, the two latter ones are loaded with sirolimus. Independent of the stent, the adventitial inflammation was associated with the adventitial area (P = 0.006 8) and the inflammation score (P = 0.037 1); and the adventitial actin-positive cells with the vascular damage (P = 0.001 2). A significant relationship was observed between the greater percentages of the restenosis and the more intense inflammation (P = 0.035 1) and the actin-positive cells (P = 0.011 9) in the adventitia. The polymeric vehicle increased the adventitial actin-positive cells (P = 0.018), independent of the type of polymer. The adventitial changes seem to be related to the restenosic process 28 days after the coronary stenting. Further investigations are necessary to confirm the role of the polymeric vehicle on the adventitial histopathological changes.

Keywords: adventitia; pig; polymer; restenosis; stent

A percutaneous transluminal coronary angioplasty is a commonplace treatment to resolve a vascular occlusion due to atherosclerosis; however, vascular repair following stent implantation causes a cel-

https://doi.org/10.17221/159/2019-VETMED

lular response that raises the risk of restenosis (Perez de Prado et al. 2013). The use of drug-eluting stents (DES) has been a major advance because it has reduced the rate of restenosis and the incidence of repeated revascularisations (Tomberli et al. 2018). Nevertheless, the first-generation DES were linked to an increased risk of stent thrombosis and late restenosis (Joner et al. 2006). These negative results have been associated with a chronic arterial inflammatory response due to permanent presence of the polymer (van der Giessen et al. 1996; Virmani et al. 2004; Natsuaki et al. 2013). Extensive research has been performed to obtain new types of DES with biocompatible polymers, biodegradable polymers, polymer-free, and fully bioresorbable scaffolds (McMahon et al. 2018; Tomberli et al. 2018). DES with biodegradable polymers eliminate one of the stimuli that causes the persistent inflammation in the vessel (Joner et al. 2006). However, some studies have found that degradation of the matrix polymer also releases reaction products that cannot be metabolised safely (Borhani et al. 2018).

More preclinical investigations are necessary to clarify the doubts arising around these devices (Perkins and Rippy 2019). To perform this type of study, coronary arteries from swine are the model of choice because of their similarity to human anatomy and their suitability for analysing the restenosis induced after coronary stenting (Perez de Prado et al. 2013). The analysis of each vascular layer in the repair process after stent placement is important for the appropriate interpretation of a device's performance (Perkins and Rippy 2019). The first studies attributed a key role in the healing process to the tunica intima (Schwartz et al. 1995). However, it has been shown that the adventitia seems to be the primary early site for vessel wall response to arterial injury that occurs on the luminal side (Buccheri et al. 2016). Detailed information about the media and (neo)intimal response to coronary stent implantation can be found elsewhere (Estevez-Loureiro et al. 2015; Perez de Prado et al. 2017). The precise role of the adventitial layer in modulating the neointimal response after coronary stenting has not been precisely established and it is controversial (Fleenor and Bowles 2009; Chang et al. 2015). The aims of this study were to describe the adventitial response of porcine coronary arteries at 28 days by histomorphometric criteria and immunohistochemistry as a component of the neointimal formation and arterial remodelling after coronary stenting; and to analyse the role of the polymer used in relation to the adventitial response development after the DES placement.

MATERIAL AND METHODS

Animal model

This randomised controlled experimental trial with a final blind analysis used 15 Large White domestic female pigs (2 months old; weight of 25 ± 3 kg). All the procedures were conducted in accordance with the Spanish legal regulations (Royal Decree 53/2013) and the European Directive 2010/63EC. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of León (23/5/12).

All the surgical procedures were performed under anaesthesia, and all efforts were made to minimise suffering. The predetermined follow-up period was 1 month, the time point recommended by consensus documents to quantitate neointimal hyperplasia in the preclinical studies (Schwartz et al. 2008). The anaesthesia protocol and the surgical and intravascular procedures were performed as described previously (Estevez-Loureiro et al. 2015).

Based in our previous experience (Estevez-Loureiro et al. 2015; Perez de Prado et al. 2017) and the vessel size measured, we used 3.00 mm diameter stents with different implantation pressures to achieve 1.2 stent to artery ratio. To implant the devices, we selected the best location out of the 3 epicardial coronary arteries. Each animal received 3 different coronary stents in a randomised manner, one in each major coronary artery (left anterior descending, left circumflex artery, and right coronary artery).

Devices analysed

The following devices were analysed:

- Control stent (n = 15): L605 chromium-cobalt alloy stent, strut thickness of 80 µm.
- Biostable-polymer stent (n = 15): based on a control stent, coated with a permanent polyacrylate polymer and loaded with sirolimus.
- Biodegradable-polymer stent (n = 15): based on a control stent, coated abluminally with a 4 to 5 µm biodegradable poly(D,L)-lactic-co-glycolic polymer, loaded with sirolimus.

https://doi.org/10.17221/159/2019-VETMED

All the materials were provided by iVascular (Barcelona, Spain). The analysis and interpretation of the results were conducted independently of the sponsor.

Tissue processing

Once the 28-day follow-up period was completed, the animals were sedated and euthanised by a T61 intravenous agent (Intervet, Salamanca, Spain). The stented segments were processed according to the previously published technique (Estevez-Loureiro et al. 2015). Sections of the central stented segment were cut with a diamond blade into 7 µm-thick slices and stained with Haematoxylin&Eosin (H&E) and Verhoeff-van Gieson (VVG).

The histopathological evaluation was performed by a veterinary pathologist blind to the type of stent.

Vascular injury and inflammation

The endothelial coverage was semi-quantified and expressed as an estimated percentage of the lumen circumference (Sperling et al. 2019). The injury score (IS) for each stent implantation site (strut) was assessed in the VVG-stained sections using a semi-quantitative method (Schwartz et al. 1992). The extent of the inflammatory changes around each stent strut (inflammation score) was assessed in the H&E-stained sections using a semi-quantitative scale (Kornowski et al. 1998). The individual scores for the struts of each stent were recorded and then averaged to obtain the mean IS and inflammation score per stented segment. The adventitial inflammation was assessed according to a semi-quantitative score (Hofma et al. 1998).

Quantitative histomorphometry

The VVG-stained arteries were analysed histomorphometrically using an Olympus PRovis AX70W digital microscope (Tokyo, Japan) with a Nikon DXM 1200W digital camera and the ImageJ-NIH Image 1.4 software package (National Institute of Health, USA). Planimetry was used to determine: the cross-sectional areas of the lumen; the internal elastic lamina; the external elastic lamina and the whole vessel. The whole vessel area was defined by the area of transition from the dense collagen to the loose connective tissue admixed with the epicardial adipose tissue (Sangiorgi et al. 1999). The Verhoeff's stain was used to define the transition from the dense collagen (dark pink) to the loose connective tissue (clear pink). It was, thereby, used to calculate the variables:

- %Restenosis: 100 × (1 – lumen area/internal elastic lamina area).
- Adventitial area (mm^2) = whole vessel area – external elastic lamina area.
- Adventitial area (%) =100 × (whole vessel area – external elastic lamina area)/external elastic lamina area.

Immunohistochemistry

The immunohistochemical labelling was performed on 7 µm resin section slides using the avidin-biotin-peroxidase complex method (Peroxidase Standard, Vectastain, ABC kit; Vector Laboratories, Burlingame, CA, USA). To stain the endothelial cells, a polyclonal rabbit anti-human von Willebrand factor antibody (DAKO, Glostrup, Denmark; A 0082; 1 in 500 dilution) was applied. The myofibroblasts/smooth muscle cells were detected by applying a monoclonal mouse anti-human alpha-smooth muscle actin (SMA) antibody (DAKO; clone 1A4, 1 in 50 dilution). Both of the primary antibodies were incubated overnight at 4 °C.

The presence and localisation of peroxidase were determined with diaminobenzidine (Substrate kit for peroxidase; Vector Laboratories, Burlingame, CA, USA) incubation. Sections from the arteries of the pigs were included as positive controls. Negative controls were performed by substituting the primary antibody with an irrelevant type specific antibody.

The microvessel density was expressed as the number of microvessels identified by the anti-von Willebrand factor antibody divided by the adventitial area (mm^2).

The semi-quantitative analysis regarding the presence of SMA-positive cells per strut in the adventitia was evaluated using the following scale: 0 = none; 1 = scarce; 2 = moderate; and 3 = high.

The individual scores of the struts were averaged to obtain the mean value per stented segment (I SMA).

https://doi.org/10.17221/159/2019-VETMED

Statistical analysis

The values are expressed as percentages and as the mean ± standard deviation, depending on the type of variable (recommended by the consensus documents for preclinical stents) (Schwartz et al. 2008). The differences between the mean of the groups were analysed using a Student's *t*-test and an analysis of variance. For multiple comparisons, a post hoc analysis was conducted using Dunnett's method for comparison with the control stent (CS) and using Tukey's method for the comparison of all groups. The semi-quantitative variables were analysed using the chi-square test or Fisher's exact method. All the analyses were conducted with the JMP v10 statistical software package (SAS Institute Inc., Cary, NC, USA) using a P-value < 0.05 as a cut-off for the statistical significance while the differences at $P < 0.10$ were described as tendencies.

RESULTS

In this study, 45 stents were implanted in 45 porcine coronary arteries and oversized to a mean stent/artery ratio of 1.34 ± 0.15, with no significant differences between the stents. No procedural complications or protocol deviations were observed. The animals completed the follow-up without problems. All the treated segments were permeable in the final angiographic analysis. At necropsy, no significant abnormalities were observed.

Twenty-eight days after the stent implantation, all the stents showed good re-endothelialisation (> 95%) and no thrombus was seen at the site of the implant. The inflammatory reaction of the adventitia in the stents varied from no response (Figure 1A and 1B) to an intense response (Figure 1C and 1D), and it was predominantly composed of lymphocytes and macrophages. The intensity of this inflammatory reaction only showed a statistically significant relationship with the adventitial area and the inflammatory response around the stent (inflammation score), but not with the microvessel density, I SMA and the IS (Table 1). This adventitial inflammatory response tended to be positively correlated to the percentage of restenosis developed (Table 1). Although there was an increase in the percentage of the restenosis in relation to the degrees of the adventitial inflammation, this difference was only significant between the no response (Figure 1A and 1B) and the more

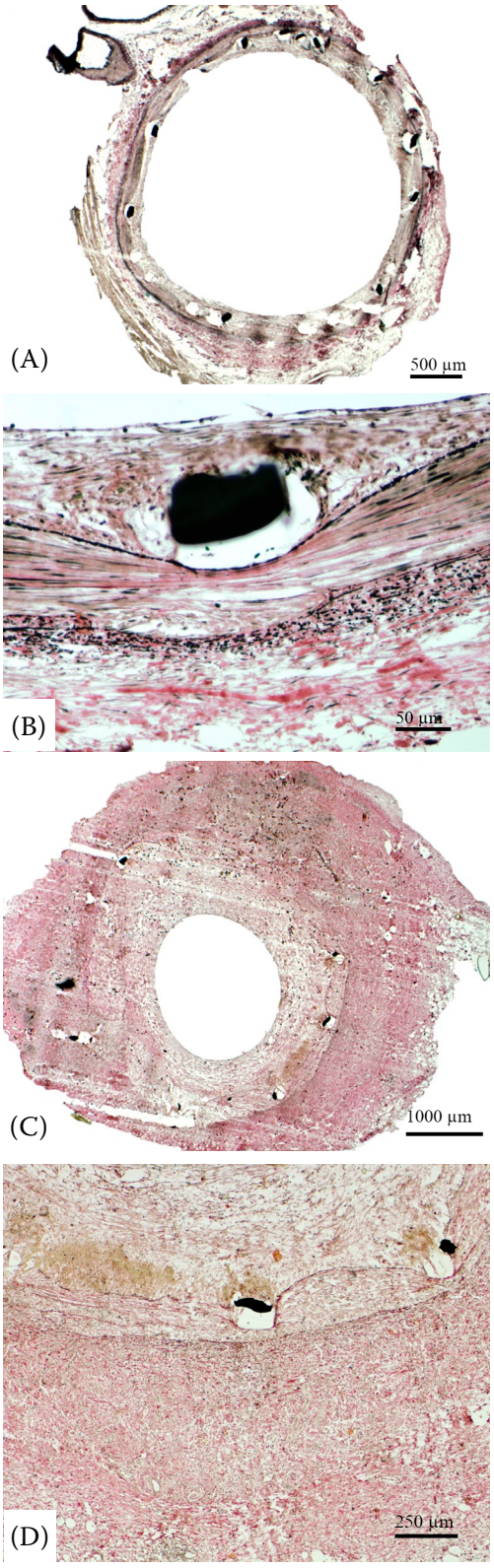

Figure 1. (A) No inflammatory response in the adventitia with a low restenosis rate. (B) Higher magnification of Figure 1A. (C) Intense adventitial inflammatory response and high rate of restenosis. (D) Higher magnification of Figure 1C. Verhoeff-van Gieson stain

https://doi.org/10.17221/159/2019-VETMED

Table 1. Relationship between the adventitial inflammation and vascular morphological parameters 28 days after the stent implantation

Adventitial inflammation	Adventitial area (%)	Microvessel density (mm^2)	I SMA	Injury score	%Restenosis	Inflammation score
0	27 ± 9	47.43 ± 20.45	0.54 ± 0.57	0.48 ± 0.09	27.09 ± 3.37	0.49 ± 0.07
1	33 ± 10	41.61 ± 19.10	0.73 ± 0.61	0.71 ± 0.11	32.45 ± 4.08	0.45 ± 0.08
2	40 ± 8	54.39 ± 27.08	0.84 ± 0.43	0.80 ± 0.17	42.98 ± 6.46	0.83 ± 0.12
P-value	0.006 8	0.447 2	0.446 8	0.123 8	0.099 0	0.037 1

I SMA = semi-quantitative value related to the presence of the adventitial SMA-positive cells; inflammation score = inflammatory changes around the struts per stented segment

Data are shown as the mean ± SD

intense inflammatory degree (Figure 1C and 1D) (P = 0.035 1). In addition, the presence of adventitial SMA-positive cells was significantly associated to the IS (P = 0.001 2) and to the greater values of restenosis (P = 0.011 9; Figure 2A–D). Finally, the results did not show any correlation between the percentage of the restenosis and the adventitial area (P = 0.172 8).

Role of the polymeric vehicle in the adventitial response

No differences between the IS and the type of stent analysed were found (Table 2), which suggests that the possible differences in the adventitial response of each treatment were not due to the degree of the lesion caused in the vessel.

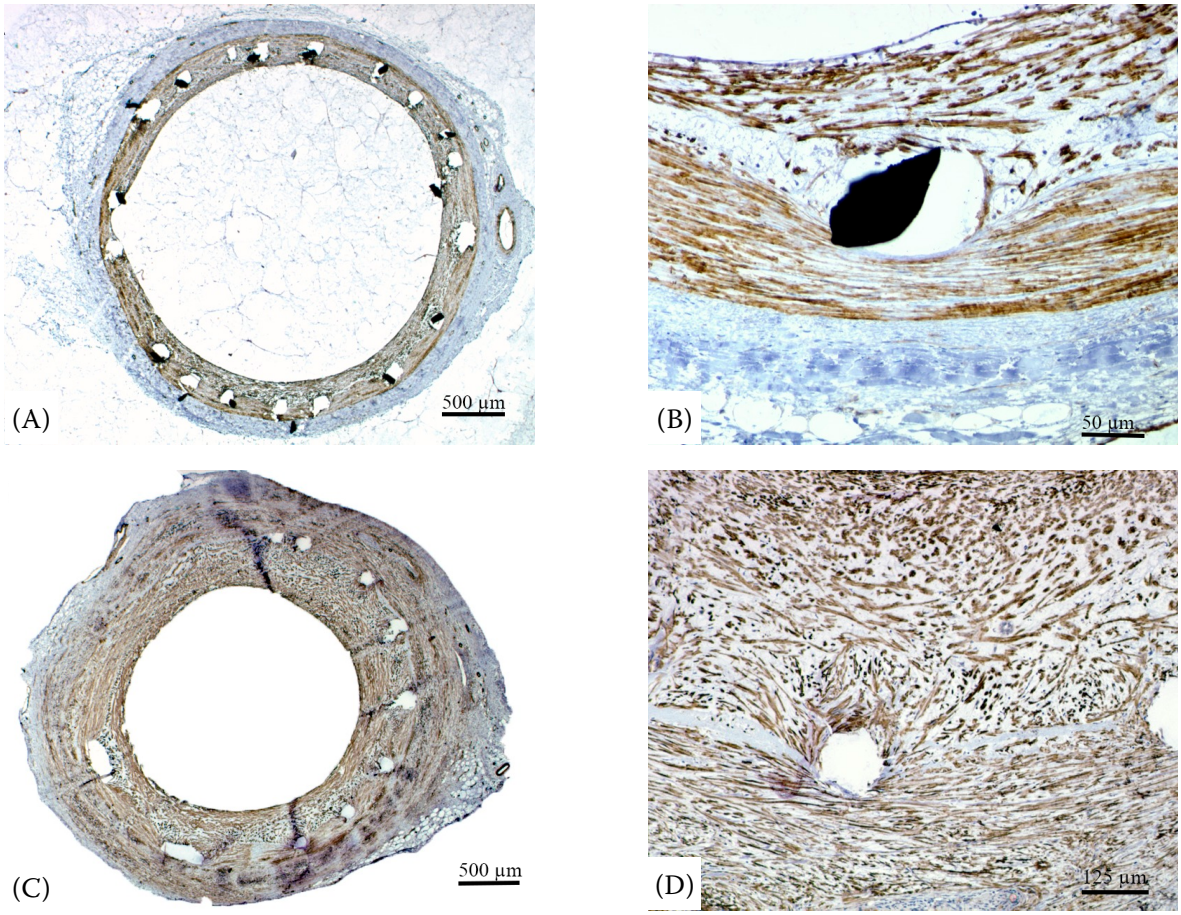

Figure 2. (A) Scarce adventitial SMA-positive cells in a stented artery with IS 0. (B) Higher magnification of Figure 2A. (C) Numerous adventitial SMA-positive cells in a stented artery with IS > 0. (D) Higher magnification of Figure 2C. Anti-alpha-smooth muscle actin (ABC-P)

https://doi.org/10.17221/159/2019-VETMED

Table 2. Vascular morphological parameters 28 days after implantation in each type of analysed stent

Stent	Injury score	Adventitial area (%)	Adventitial inflammation	Microvessel density (mm^2)	I SMA
CS	0.53 ± 0.48	29 ± 11	0.40 ± 0.63	50.59 ± 17.89	0.35 ± 0.42
BS	0.54 ± 0.28	31 ± 10	0.60 ± 0.74	40.42 ± 17.62	0.79 ± 0.39
BD	0.73 ± 0.45	33 ± 9	0.92 ± 0.76	47.76 ± 26.78	0.87 ± 0.65
P-value	0.323 6	0.547 8	0.161 4	0.433 9	0.018 4

BD = biodegradable-polymer stent; BS = biostable-polymer stent; CS = control stent; I SMA = semi-quantitative value related to the presence of the adventitial SMA-positive cells

Data are shown as the mean ± SD

The polymeric vehicle increased only the number of adventitial alpha-SMA cells ($P = 0.018$), although no differences were detected according to the polymer type (Table 2).

DISCUSSION

Numerous investigations describe the role of the adventitia in the development of restenosis after a balloon angioplasty (Shi et al. 1996; Fleenor and Bowles 2009; Chang et al. 2015); however, few studies have evaluated this vascular layer after a stent implantation (Hofma et al. 1998; Nishimiya et al. 2015).

Twenty-eight days after the coronary stenting, the adventitial inflammation was related to the inflammatory response around the stent, as also cited by other authors (Maiellaro and Taylor 2007), but not with the microvessel density unlike other studies (Nishimiya et al. 2015), perhaps due to the small sample size. Nowadays, neointima formation after a percutaneous transluminal coronary angioplasty is considered to be conditioned mainly by two factors: one is dependent on the angiogenesis (Mulligan-Kehoe and Simons 2014), and the other is related to the migration of the muscle cells and adventitial myofibroblasts (Buccheri et al. 2016). In this study, we observed that the increase in the adventitial SMA-positive cells was associated with a greater IS. This finding has been linked to the transformation of adventitial fibroblasts in myofibroblasts (SMA-positive cells) after a vascular injury (Shi et al. 1996). Therefore, it seems critical to control the depth of the damage after the stent implantation to impact on one of the cell sources that have the ability to migrate to the neointima and maintain the restenosic process (Shi et al. 1996; Christen et al. 2001).

In this sense, we observed a relationship between the adventitial SMA-positive cells and the percentage of the restenosis. Thus, the inhibition of SMA-positive cells either by controlling the IS or by specific drugs that act on these cells could be a target for the prevention and treatment of a negative vascular remodelling (Goel et al. 2012). All these data seem to support the hypothesis that the adventitia could act as a functional homeostatic regulator in the setting of the restenosis after a percutaneous coronary angioplasty (Stenmark et al. 2013). The materials used in the DES have been shown to cause an inflammatory response in a variable degree, so the choice of a suitable polymer can help to minimise this inflammation and to facilitate healing within the stented artery (Borhani et al. 2018). Thus, a second objective of our work was to analyse the effect that the nature of the polymeric vehicle could have on the histopathological changes in the adventitia after the coronary stenting, as a component of the vascular response (Stenmark et al. 2013). We could not demonstrate that the presence and the type of polymer caused changes in the adventitial inflammatory reaction or microvessel density as cited by van der Giessen et al. (1996) and Nishimiya et al. (2015), perhaps due to the relatively small sample size. However, we observed a greater presence of SMA-positive cells in the stents with polymers versus the control stents. One possible explanation for this finding could be a higher IS; however, no significant differences were found with the polymer-free stents. Further investigations are required to confirm the significance of the adventitial SMA-positive cells in the stents with a polymeric vehicle. Besides, we would expect that as the polymer degrades, the vascular response would become more similar to a control stent than to a non-biodegradable polymer-coated stent; however, no differences were detected between the polymer-coated stents. Further studies are necessary to assess this point.

https://doi.org/10.17221/159/2019-VETMED

The major findings in this study were:

(1) The adventitial inflammation was related to the adventitial area and inflammation score; (2) the adventitial SMA-positive cells were associated with the vascular damage (IS); (3) the greater percentages of the restenosis were significantly related to the more intense adventitial inflammatory response and the presence of SMA-positive cells in the adventitia; and (4) the polymeric vehicle, regardless of the type, increased the number of adventitial SMA-positive cells.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Borhani S, Hassanajili S, Ahmadi Tafti SH, Rabbani S. Cardiovascular stents: Overview, evolution, and next generation. Prog Biomater. 2018 Sep;7(3):175-205.

Buccheri D, Piraino D, Andolina G, Cortese B. Understanding and managing in-stent restenosis: A review of clinical data, from pathogenesis to treatment. J Thorac Dis. 2016 Oct;8(10):E1150-62.

Chang H, Lei H, Zhao Y, Yang R, Wu A, Mao Y, Huang Y, Lv X, Zhao J, Lou L, Zhang D, He Y, Xu Y, Yang T, Zhao M. Yiqihuoxuejiedu formula restrains vascular remodeling by reducing the inflammation reaction and Cx43 expression in the adventitia after balloon injury. Evid Based Complement Alternat Med. 2015;2015:904273.

Christen T, Verin V, Bochaton-Piallat M, Popowski Y, Ramaekers F, Debruyne P, Camenzind E, van Eys G, Gabbiani G. Mechanisms of neointima formation and remodeling in the porcine coronary artery. Circulation. 2001 Feb 13;103(6):882-8.

Estevez-Loureiro R, Perez de Prado A, Perez-Martinez C, Cuellas-Ramon C, Regueiro-Purrinos M, Gonzalo-Orden JM, Lopez-Benito M, Molina-Crisol M, Duocastella-Codina L, Fernandez-Vazquez F. Safety and efficacy of new sirolimus-eluting stent models in a preclinical study. Rev Esp Cardiol (Engl Ed). 2015 Dec;68(12):1118-24.

Fleenor BS, Bowles DK. Negligible contribution of coronary adventitial fibroblasts to neointimal formation following balloon angioplasty in swine. Am J Physiol Heart Circ Physiol. 2009 May;296(5):H1532-9.

Goel SA, Guo LW, Liu B, Kent KC. Mechanisms of post-intervention arterial remodelling. Cardiovasc Res. 2012 Dec 1;96(3):363-71.

Hofma SH, Whelan DM, van Beusekom HM, Verdouw PD, van der Giessen WJ. Increasing arterial wall injury after long-term implantation of two types of stent in a porcine coronary model. Eur Heart J. 1998 Apr;19(4):601-9.

Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, Kutys R, Skorija K, Gold HK, Virmani R. Pathology of drug-eluting stents in humans: Delayed healing and late thrombotic risk. J Am Coll Cardiol. 2006 Jul 4;48(1):193-202.

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 Jan;31(1):224-30.

Maiellaro K, Taylor WR. The role of the adventitia in vascular inflammation. Cardiovasc Res. 2007 Sep;75(4):640-8.

McMahon S, Bertollo N, O'Cearbhaill ED, Salber J, Pierucci L, Duffy P, Durig T, Bi V, Wang W. Bio-resorbable polymer stents: A review of material progress and prospects. Prog Polym Sci. 2018;83:79-96.

Mulligan-Kehoe MJ, Simons M. Vasa vasorum in normal and diseased arteries. Circulation. 2014 Jun 17;129(24):2557-66.

Natsuaki M, Kozuma K, Morimoto T, Kadota K, Muramatsu T, Nakagawa Y, Akasaka T, Igarashi K, Tanabe K, Morino Y, Ishikawa T, Nishikawa H, Awata M, Abe M, Okada H, Takatsu Y, Ogata N, Kimura K, Urasawa K, Tarutani Y, Shiode N, Kimura T; NEXT Investigators. Biodegradable polymer biolimus-eluting stent versus durable polymer everolimus-eluting stent: A randomized, controlled, noninferiority trial. J Am Coll Cardiol. 2013 Jul 16;62(3):181-90.

Nishimiya K, Matsumoto Y, Shindo T, Hanawa K, Hasebe Y, Tsuburaya R, Shiroto T, Takahashi J, Ito K, Ishibashi-Ueda H, Yasuda S, Shimokawa H. Association of adventitial vasa vasorum and inflammation with coronary hyperconstriction after drug-eluting stent implantation in pigs in vivo. Circ J. 2015;79(8):1787-98.

Perez de Prado A, Perez-Martinez C, Cuellas C, Gonzalo-Orden JM, Diego A, Regueiro M, Martinez-Fernandez B, Altonaga JR, Marin Francisco JG, Fernandez-Vazquez F. Preclinical evaluation of coronary stents: Focus on safety issues. Curr Vasc Pharmacol. 2013 Jan;11(1):74-99.

Perez de Prado A, Perez Martinez C, Cuellas Ramon C, Regueiro Purrinos M, Lopez Benito M, Gonzalo Orden JM, Rodriguez Altonaga JA, Estevez Loureiro R, Benito Gonzalez T, Vinuela Baragano D, Molina Crisol M, Amoros Aguilar M, Perez Serranos I, Vidal Parreu A, Benavides Montegordo A, Duocastella Codina L, Fernandez Vazquez F. Safety and efficacy of new biodegradable polymer-based sirolimus-eluting stents in a preclinical model. Rev Esp Cardiol (Engl Ed). 2017 Dec;70(12):1059-66.

https://doi.org/10.17221/159/2019-VETMED

Perkins LEL, Rippy MK. Balloons and stents and scaffolds: Preclinical evaluation of interventional devices for occlusive arterial disease. Toxicol Pathol. 2019 Apr;47(3): 297-310.

Sangiorgi G, Taylor AJ, Farb A, Carter AJ, Edwards WD, Holmes DR, Schwartz RS, Virmani R. Histopathology of postpercutaneous transluminal coronary angioplasty remodeling in human coronary arteries. Am Heart J. 1999 Oct;138(4 Pt 1):681-7.

Schwartz SM, Majesky MW, Murry CE. The intima: Development and monoclonal responses to injury. Atherosclerosis. 1995 Dec;118 Suppl:S125-40.

Schwartz RS, Huber KC, Murphy JG, Edwards WD, Camrud AR, Vlietstra RE, Holmes DR. Restenosis and the proportional neointimal response to coronary artery injury: Results in a porcine model. J Am Coll Cardiol. 1992 Feb; 19(2):267-74.

Schwartz RS, Edelman E, Virmani R, Carter A, Granada JF, Kaluza GL, Chronos NA, Robinson KA, Waksman R, Weinberger J, Wilson GJ, Wilensky RL. Drug-eluting stents in preclinical studies: Updated consensus recommendations for preclinical evaluation. Circ Cardiovasc Interv. 2008 Oct;1(2):143-53.

Shi Y, O'Brien JE, Fard A, Mannion JD, Wang D, Zalewski A. Adventitial myofibroblasts contribute to neointimal formation in injured porcine coronary arteries. Circulation. 1996 Oct 1;94(7):1655-64.

Sperling C, Waliszewski MW, Kherad B, Krackhardt F. Comparative preclinical evaluation of a polymer-free sirolimus-eluting stent in porcine coronary arteries. Ther Adv Cardiovasc Dis. 2019 Jan-Dec;13:1753944719826335.

Stenmark KR, Yeager ME, El Kasmi KC, Nozik-Grayck E, Gerasimovskaya EV, Li M, Riddle SR, Frid MG. The adventitia: Essential regulator of vascular wall structure and function. Annu Rev Physiol. 2013;75:23-47.

Tomberli B, Mattesini A, Baldereschi GI, Di Mario C. A brief history of coronary artery stents. Rev Esp Cardiol (Engl Ed). 2018 May;71(5):312-9.

Virmani R, Guagliumi G, Farb A, Musumeci G, Grieco N, Motta T, Mihalcsik L, Tespili M, Valsecchi O, Kolodgie FD. Localized hypersensitivity and late coronary thrombosis secondary to a sirolimus-eluting stent: Should we be cautious? Circulation. 2004 Feb 17;109(6):701-5.

van der Giessen WJ, Lincoff AM, Schwartz RS, van Beusekom HM, Serruys PW, Holmes DR Jr, Ellis SG, Topol EJ. Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. Circulation. 1996 Oct 1;94(7):1690-7.

Received: November 12, 2019
Accepted: September 16, 2020