Original Article

Origin of Restenosis after Drug-Eluting Stent Implantation in Hyperglycemia is Inflammatory Cells and Thrombus

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Aims: The cellular and molecular mechanisms and safety after drug-eluting stent (DES) implantation in diabetic patients are still poorly understood; therefore, in this study, we evaluated the pathologic responses of the sirolimus-eluting stent (SES) or paclitaxel-eluting stent (PES) in a type I diabetes mellitus (DM) rat model.

Methods: The type I DM rat model was manipulated by intra-peritoneal streptozotocin injection. Two weeks later, DES was implanted in the aorta of rats with hyperglycemia or not as a control. Four weeks after DES implantation, the stented aorta was isolated and histomorphometric analysis was performed.

Results: On histomorphometric analysis, increased thrombus, inflammatory cell infiltration, and neointimal hyperplasia (NIH) without change of the smooth muscle cell number after DES implantation were observed in DM rats compared with non-DM (NDM) rats. Furthermore, delayed coverage of mature endothelial cells defined as a von Willebrand factor expression and increased immature endothelial cells as a c-kit expression after DES implantation were observed in DM rats compared with NDM rats. Increased fibrin deposition and decreased hyaluronic acid accumulation at NIH after DES implantation were also observed in DM rats compared with NDM rats.

Conclusions: In conclusion, the main mechanism of restenosis after DES implantation under hyperglycemic conditions was initial thrombus with changes of the extracellular matrix rather than SMC proliferation. These results provided a therapeutic clue for the selection of DES and application of combination therapy using anti-thrombotic and anti-inflammatory drugs in diabetic patients.

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Key words; Drug-eluting stents, Type 1 diabetes mellitus, Restenosis

Introduction

Coronary artery revascularization in patients with type 1 and type 2 diabetes mellitus (DM) continues to be a challenge. Patients with DM suffer from a higher rate of repeated revascularization and worse outcome after percutaneous coronary intervention (PCI) compared with the general population^{1,2}. Data

after balloon angioplasty or bare metal stent (BMS) implantation have demonstrated higher rates of death and stent thrombosis (ST) in diabetic than in non-diabetic patients³⁻⁵.

The increased atherothrombotic risk in patients with DM is related to their pro-inflammatory and pro-thrombotic status. Platelets from diabetic subjects show increased adhesiveness and exaggerated aggregation. Reduced responsiveness of patients with DM to anti-platelet therapy has also been documented^{5,6}.

Previously, in patients with acute myocardial infarction (AMI), coronary artery plaques of diabetic patients had significantly higher necrotic core percentages and lower fibro-fatty tissue percentages than non-

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diabetic patients^{6, 7}). In patients with stable angina, dense calcium in culprit lesions was significantly larger in diabetic patients than non-diabetic patients by analysis using virtual histology intravascular ultrasound (VH-IVUS)^{7, 8}).

The introduction of drug-eluting stents (DES) has improved clinical outcomes in diabetic patients. Although DES is now widely used, only limited data are available for systematic evaluation of their safety in this specific population. Furthermore, recent data have raised concerns about increased risks of ST and mortality over longer periods of follow-up after DES implantation. In diabetic patients, subgroup analysis of recent registries of DES implantation has already suggested that DM might be a risk factor for ST⁹).

The over all rate of ST after DES implantation was ~0.6%, and did not differ between sirolimus-eluting stent (SES) and paclitaxel-eluting stent (PES). This incidence is comparable to that of BMS¹⁰; however, the duration of thienopyridine treatment tended to be longer with SES or PES; thus, higher thrombogenicity of SES or PES that might have been mitigated by a longer duration of dual anti-platelet treatment cannot be ruled out¹¹).

Almost all previous reports were performed in a clinical setting without any clear histomorphometric analysis. Little basic research using rat or mouse DM models has been performed using short BMS of 7 mm¹²) or 9 mm length¹³), and only one report used SES in a large animal model of DM¹⁴).

With no confirmed evidence, the pathogenic mechanism of restenosis after DES implantation under hyperglycemia is still not completely understood; therefore, we elucidated the histologic vascular responses after DES implantation using SES and PES in a streptozotocin-induced type 1 DM rat model.

Methods

Animal Care and Induction of Diabetic Mellitus

Male Sprague Dawley (SD) rats were purchased from Samtako, Inc. (Daejeon, Korea) at 8 weeks of age for the experiments, and allowed to grow until 10-12 weeks. All rats were fed a normal pellet diet and given water ad libitum. Animals were fasted for 18-24 hours and streptozotocin (STZ; 65 mg/kg, Sigma-Aldrich Inc, MO, USA) was administered intraperitoneally. The age-matched control group received citrate buffer only. Blood glucose levels were monitored every week using an Accu-check blood glucose meter (Roche Diagnostics, Basel, Switzerland). To control the blood glucose, DM rats received insulin (2 units/kg) daily. Rats with a stable blood glucose level ≥ 300 mg/dL for

2 consecutive weeks were considered diabetic. The rats' drinking water contained aspirin (3.25 mg/kg; Bayer Vital GmbH, Germany) from 3 days before stent implantation daily throughout the study period.

All experiments were conducted in accordance with the institutional guidelines for the use and care of laboratory animals which conforms with 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). Also, animal protocols were approved by the Chonnam National University Animal Care and Use Committee (No = CNU IACUC-H-2009-16).

Stent Deployment and Aortogram

For stent implantation, streptozotocin-induced DM rats or non-DM (NDM) rats were anesthetized with an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (6.7 mg/kg), and the left common carotid artery was surgically exposed. Under fluoroscopic guidance, the stent delivery system was advanced to the mid-aorta via a left common carotid arteriotomy. In the experiment, a paclitaxel-eluting stent (PES) ($n=4$; TAXUS Liberte 2.75×16 mm; Boston Scientific, MA, USA), sirolimus eluting stent (SES) ($n=4$; CYPHER select 2.75×16 mm; Cordis Corporation, NV, Netherlands), or bare metal stent (BMS) ($n=4$; Maneo $2.3.0 \times 19$ mm; DEVON Medical, Menden, Germany) was deployed to around 3.0 mm of the stent-inflated diameter in both DM and non-DM individually.

Tissue Preparation and Histomorphometric Analysis

After the final aortogram, the aorta was flushed gently with 0.9% saline solution and perfusion fixation was performed with 2.5% glutaraldehyde. The stented aorta was collected with at least 1 cm of the proximal and distal host vessel attached. After an additional 24 hours of immersion fixation with 2.5% glutaraldehyde, the stented aorta was cut into two segments. One segment was divided horizontally and gross findings were checked in one part. The other part was used for scanning electron microscopy (SEM, S-3500N scanning electron microscope; Hitachi Co., Japan). SEM analysis was conducted according to the standard procedures as previously described¹⁵). After gross examination, one part was fixed with 4% formalin. After 24 hours, the stent strut was removed and was processed for immunohistochemistry and specific-staining.

Immunohistochemistry was conducted following standard procedures as previously described¹³). Anti-vWF monoclonal antibody (Sigma), anti-smooth

Fig. 1. Gross findings and surface electron microscopy (SEM) analysis of stent-implanted aorta. (A) Representative pictures of gross findings. (B) Representative pictures of SEM in low power field. (C) Representative pictures of SEM in high power field of DM-SES. (D) Representative pictures of SEM in high power field of DM-PES. C: covering structure by fibrin, T: thrombus with red blood cells, S: stent strut. NDM: non-diabetes mellitus, DM: diabetes mellitus, SES: sirolimus-eluting stent, PES: paclitaxel-eluting stent, BMS: bare metal stent. Green arrow: thrombus

muscle actin (SMA) monoclonal Ab (Sigma) and anti-CD 68 Ab (BMA BIOMEDICALS, Augst, Switzerland) were used. The anti-vascular cell adhesion molecule (VCAM) Ab, anti-c-kit Ab and plasminogen activator inhibitor-1 (PAI-1) Ab were purchased from Santa Cruz Biotechnology inc (CA, USA). All sections were analyzed by conventional light microscopy, digital photography or fluorescence microscopy. Fibrin deposition and hyaluron accumulation were stained specifically.

The other segment was placed in 10% formalin for an additional 24 hours. Cross-sections were prepared from the segment by embedding the tissue in acrylic plastic and cutting the total block. All sections were stained with hematoxylin and eosin (H&E stain). Histomorphometric analysis was carried out using the Nis-Elements version 3.00 SP7 (Nikon Co., Tokyo, Japan). Neointimal hyperplasia (NIH) was evaluated in each tissue cross-section, averaged, and expressed as the absolute area in μm^2 . Additionally, focal thrombo-

Fig. 2. Focal thrombosis and covering stent strut ratio.

(A) Bar graph of focal thrombosis ratio. (B) Representative picture of internalized thrombus at neo-intimal hyperplasia in H&E stained slide. (C) Bar graph of covering stent strut ratio. Representative pictures of covered stent strut (D) and uncovered stent strut (E) in H&E-stained slide.

NDM: non-diabetes mellitus, DM: diabetes mellitus, SES: sirolimus-eluting stent, PES: paclitaxel-eluting stent, BMS: bare metal stent. Green arrow: internalized thrombus, blue arrow: covered stent strut, red arrow: uncovered stent strut

sis was detected in a microscopic high power field. We defined the thrombus-detected strut as being attached to the thrombus in the NIH area around the stent strut and calculated the thrombus-detected strut/total number of struts ratio (%) in one section of the slide. The covering strut ratio was calculated by the number of exposed struts/number of total struts ratio (%) in the lumen by microscopic observation. Covering struts and uncovering struts were defined in high power field by microscopic observation. These were

averaged over all tissue cross-sections slides with H&E staining.

Statistical Analysis

Measurements of each stented vessel (total sections from mid-to distal stent) were averaged to produce a mean value per stent. Tests of significance were two-tailed, and significance was established by a value of $p < 0.05$. * is the symbol of p value ($p < 0.05$) with SES or PES group vs BMS group in NDM or DM

Table 1. Blood glucose level of non-diabetic and diabetic rats

	0 day	1 week	2 weeks	3 weeks	4 weeks
NDM-Sham	129.0 ± 8.5	127.0 ± 7.1	121.0 ± 1.4	125.5 ± 12.0	147.0 ± 8.5
NDM-PES	189.3 ± 6.0	128.7 ± 5.8	114.3 ± 4.7	116.0 ± 2.6	128.0 ± 11.5
NDM-SES	125.7 ± 8.3	125.3 ± 5.8	116.0 ± 8.7	128.3 ± 9.8	144.7 ± 7.2
NDM-BMS	131.3 ± 9.0	124.3 ± 6.8	122.3 ± 9.5	128.7 ± 5.8	140.3 ± 16.0
NDM-AVE	143.8 ± 30.4	126.3 ± 1.9	118.4 ± 3.9	124.6 ± 5.9	140.0 ± 8.5
DM-Sham	405.0 ± 7.1	422.5 ± 3.5	420.0 ± 7.1	437.5 ± 17.7	414.0 ± 19.8
DM-PES	566.7 ± 44.6	442.3 ± 96.1	574.0 ± 41.6	472.0 ± 67.8	569.3 ± 53.1
DM-SES	530.7 ± 54.0	528.0 ± 78.1	555.7 ± 76.8	430.7 ± 92.8	565.7 ± 59.5
DM-BMS	570.0 ± 38.2	491.7 ± 166.1	466.7 ± 115.5	462.7 ± 86.8	424.0 ± 75.9
DM-AVE	518.1 ± 77.5	471.1 ± 47.8	504.1 ± 73.1	450.7 ± 19.8	493.3 ± 85.8

NDM: non-diabetes mellitus, DM: diabetes mellitus, SES: sirolimus-eluting stent, PES: paclitaxel-eluting stent, AVE: average

Table 2. Body weights of non-diabetic and diabetic rats

	0 day	1 week	2 weeks	3 weeks	4 weeks
NDM	427.5 ± 3.5	442.5 ± 3.5	455.0 ± 7.1	490.0 ± 14.1	525.0 ± 35.4
NDM-PES	433.3 ± 10.4	455.0 ± 13.2	476.7 ± 23.1	511.7 ± 10.4	541.7 ± 14.4
NDM-SES	428.3 ± 2.9	450.0 ± 10.0	470.0 ± 20.0	501.7 ± 12.6	528.3 ± 20.2
NDM-BMS	435.0 ± 8.7	458.3 ± 7.6	483.3 ± 11.5	508.3 ± 16.1	518.3 ± 19.2
NDM-AVE	431.0 ± 3.7	451.5 ± 6.9	469.0 ± 11.6	502.9 ± 9.6	528.3 ± 9.8
DM	405.0 ± 7.1	415.0 ± 14.1	417.5 ± 10.6	437.5 ± 24.7	412.5 ± 17.7
DM-PES	370.0 ± 26.5	401.7 ± 37.5	416.7 ± 28.9	385.0 ± 60.6	385.0 ± 40.9
DM-SES	346.7 ± 47.3	376.7 ± 46.2	378.3 ± 40.7	345.0 ± 69.5	376.7 ± 41.9
DM-BMS	386.7 ± 32.1	396.7 ± 32.1	386.7 ± 23.1	380.0 ± 20.0	393.3 ± 25.2
DM-AVE	377.1 ± 24.8	397.5 ± 15.9	399.8 ± 20.3	386.9 ± 38.2	391.9 ± 15.3

NDM: non-diabetes mellitus, DM: diabetes mellitus, SES: sirolimus-eluting stent, PES: paclitaxel-eluting stent, AVE: average

rats and # with NDM vs DM rats at SES or PES group. Unless otherwise noted, data are reported as the mean ± standard deviation of the mean.

Results

Blood Glucose Level

Administration of streptozotocin resulted in significant elevation of blood glucose levels after 2 weeks that were sustained throughout the duration of the study (**Table 1**). On average, NDM rats had a mean blood glucose level of 140.0 ± 8.5 mg/dL and DM rats 493.3 ± 85.8 mg/dL at 4 weeks after stent implantation (**Table 1**). On average, NDM rats had a mean body weight of 431.0 ± 3.7 g and DM rats 377.1 ± 24.8 g at the time of stent implantation (**Table 2**).

Gross Findings and SEM Analysis

In **Fig. 1A**, thrombus was mainly located in the surrounding portion of the stent strut (green arrows) and was increased in DM rats compared with NDM

rats.

In **Fig. 1B**, at a lower power field of SEM analysis, uncovered stent struts were observed in both DM and NDM rats with SES and PES. A clear large thrombus was also detected in stent struts (**Fig. 1B**, green arrows). In DM rats, both SES and PES groups showed a more increased thrombus than in NDM. In **Fig. 1C**, the DM-PES group had a more structured thrombus by red blood cells with a fibrin-like sandwich form compared with the DM-SES group.

Thrombus in the DM-PES group was characterized into three parts: C: covering structure by fibrin, T: thrombus with red blood cells, S: stent strut itself (**Fig. 1D**). Red blood cells with thread fibrin attached to stent struts were covered by very thin film.

Neointimal Hyperplasia and its Cellular Components

In both SES and PES groups, NIH was significantly larger in DM rats (DM-SES: $0.57 \pm 0.09^*$, DM-PES: $0.61 \pm 0.14^*$, DM-BMS: 0.39 ± 0.08 mm²; $^*p < 0.05$, DM vs. NDM) compared with NDM rats

Fig. 3. Number of two types of cell, cell component ratio, and representative pictures of immunohistochemistry (IHC) staining using anti-smooth muscle cell (SMC) actin Ab at neointimal hyperplasia (NIH).

(A) Bar graph of total cells, SMC, and non-SMC numbers at NIH (mm²). (B) Bar graph of SMC and non-SMC ratio (%). (C) Definite methods of SMC (green-and blue color-positive cells) and non-SMC (blue-positive cells). (D) Representative picture of IHC stain using anti-SMC Ab in stent-implanted aorta. NDM: non-diabetes mellitus, DM: diabetes mellitus, SES: sirolimus-eluting stent, PES: paclitaxel-eluting stent, BMS: bare metal stent. White arrow: media, yellow arrow: NIH.

Fig. 4. Fibrin deposition and macrophage infiltration at neointimal hyperplasia (NIH).

(A) Representative pictures of fibrin deposition around the stent strut. (B) Representative pictures of macrophage infiltration, red arrow: macrophage identified by CD-68-positive cells. (C) Bar graph of macrophage number in NIH in 400 magnification field.

Yellow arrow: fibrin, red arrow: macrophage, NDM: non-diabetes mellitus, DM: diabetes mellitus, SES: sirolimus-eluting stent, PES: paclitaxel-eluting stent, BMS: bare metal stent.

(NDM-SES: 0.34 ± 0.01 mm², NDM-PES: 0.39 ± 0.03 mm², NDM-BMS: 0.38 ± 0.15 mm²).

As seen in **Fig. 2A** and **B**, the focal thrombus ratio was increased significantly after SES and PES implantation in DM rats (DM-SES: $29.1 \pm 19.7\%$ *, DM-PES: $50.8 \pm 9.0\%$ *, DM-BMS: $2.7 \pm 0.3\%$) compared with NDM rats (NDM-SES: $0.9 \pm 1.5\%$ #, NDM-PES: $22.6 \pm 4.9\%$, NDM-BMS: $0.4 \pm 0.6\%$).

As seen in **Fig. 2C**, **D** and **E**, the covering stent strut ratio did not show a difference between DM rats (DM-SES: $53.8 \pm 11.0\%$ *, DM-PES: $49.8 \pm 2.7\%$ *#, DM-BMS: $77.4 \pm 7.2\%$) and NDM rats (NDM-SES: $42.2 \pm 15.8\%$ *, NDM-PES: $61.1 \pm 4.7\%$ *, NDM-BMS: $83.0 \pm 4.4\%$); however, it was more decreased in SES and PES groups than in the BMS group in DM rats.

To analyze the characteristics of smooth muscle cells (SMC) at NIH, the stented aorta was stained by anti-SMC Ab. In **Fig. 3D**, the stented aorta was divided into media (M: white arrow) and NIH (N: yellow area). NIH was formed by SMC (green and blue fluorescence double-positive cells) and non-SMC

(only blue fluorescence-positive cells) (**Fig. 3C**). As seen in **Fig. 3A**, SMC, non-SMC, and total cell numbers were decreased in the PES group compared with the BMS group in both DM and NDM rats; however, the SES group decreased the number of these cell types only in DM rats [(Total cell numbers/mm²; NDM-SES: 2980.1 ± 147.5 , NDM-PES: 1604.9 ± 129.8 , NDM-BMS: 3346.2 ± 359.9 , DM-SES: 2284.0 ± 269.3 , DM-PES: 1571.7 ± 568.7 , DM-BMS: 3155.2 ± 224.0), (SMC numbers/mm²; NDM-SES: 1496.7 ± 139.7 , NDM-PES: 755.9 ± 77.3 , and NDM-BMS: 1569.5 ± 183.6 , DM-SES: 618.2 ± 146.8 , DM-PES: 376.1 ± 414.7 , DM-BMS: 1467.2 ± 199.8), (non-SMC numbers/mm²; NDM-SES: 1483.5 ± 7.9 , NDM-PES: 849.0 ± 135.8 , NDM-BMS: 1776.6 ± 183.4 , DM-SES: 1665.7 ± 414.6 , DM-PES: 1195.6 ± 164.7 , DM-BMS: 1688.0 ± 33.6)].

A significant change of the cell components at NIH in DM rats was observed. SES and PES groups had an increased non-SMC ratio in DM rats compared with the BMS group [(non-SMC ratio; NDM-SES: $49.9 \pm 2.2\%$, NDM-PES: $47.0 \pm 4.7\%$, NDM-

Fig. 5. Hyaluron accumulation and expression of vascular cell adhesion molecule (VCAM).

(A) Hyaluron accumulation at neointimal hyperplasia, blue: hyaluron. (B) Representative pictures of immunohistochemistry stain using anti-VCAM Ab, black arrow: VCAM-positive stained cells (brown).

NDM: non-diabetes mellitus, DM: diabetes mellitus, SES: sirolimus-eluting stent, PES: paclitaxel-eluting stent, BMS: bare metal stent.

BMS: $53.1 \pm 1.2\%$, DM-SES: $72.2 \pm 9.6\%$, DM-PES: 79.8 ± 16.0 , DM-BMS: $53.6 \pm 3.0\%$) and (SMC ratio; NDM-SES: $50.7 \pm 0.6\%$, NDM-PES: $50.6 \pm 0.8\%$, NDM-BMS: $46.1 \pm 1.4\%$, DM-SES: $27.6 \pm 9.9\%$, DM-PES: 20.9 ± 15.3 , DM-BMS: $47.1 \pm 3.3\%$).

Immunohistochemistry

Fibrin deposition surrounding the stent struts in SES and PES groups was more increased in DM rats than non-DM rats (**Fig. 4A**); however, the fibrin deposition was not different between SES and PES

groups. Macrophages as CD-68-positive cells appeared in the NIH in SES and PES groups in DM rats (**Fig. 4B**). In DM rats, increased macrophage infiltration was observed compared with non-DM rats, and this increase was more prominent in the PES group than the SES group (**Fig. 4C**).

One of the components of the extracellular matrix, hyalurona (hyaluronic acid), was detected as a blue color in the NIH and media area (**Fig. 5A**). After stenting of SES and PES, increased accumulation of hyalurona was observed in both the NIH and media

Fig. 6. Re-endothelialization of neointimal hyperplasia (NIH).

(A) Representative pictures of immunohistochemistry stain using anti-c-kit Ab. Red round image in high power field. Black arrow: c-kit-positive stained cells. (B) Representative pictures of immunohistochemistry staining using anti-von-Willebrand factor (vWF) Ab, black arrow: vWF-positive stained cells.

area.

Also, as seen **Fig. 5B**, increased VCAM-1 expression in the sub-endothelial area of NIH was observed in DM rats compared with NDM rats after stent

implantation at SES and PES.

To confirm re-endothelialization, we used anti-c-kit Ab for detect immature endothelial cells and anti-vWF Ab to mature endothelial cells. In **Fig. 6A**, c-kit-

positive cells were observed at NIH from DM rats with PES or BMS and NDM rats with SES and BMS. At high power field observation (**Fig. 6A**, red round image), c-kit-positive cells had the morphology of endothelial cells. In **Fig. 6B**, mature endothelial cells, vWF-positive cells, were observed at NIH from NDM rats with SES and PES. The mature endothelial cells were located mainly in the interspace between stent struts except in the upper portion of stent struts.

Discussion

This study elucidated histologic analysis after implantation of commercially available 1st generation DES in rats with hyperglycemia. In this study, hyperglycemia increased NIH, and this increased NIH was induced by thrombus, fibrin deposition, and inflammatory cells without an increase of vascular SMC.

This study supports that hyperglycemia is a risk factor for stent thrombosis in clinical registries from DM patients who underwent DES implantation^{9, 10}.

Coronary stent thrombosis is an issue in the DES era. Numerous reports described the occurrence of acute (<24 hours), subacute (<30 days), late (>30 days), and very late (>12 months) stent thrombosis after DES implantation. More structural thrombus was observed in DM rats compared with NDM rats and especially, thrombus attached to stent struts with fibrin cap was observed in DM rats with PES (**Fig. 1D**). According to the SEM findings, thrombus was detected both on naked stent struts and on NIH (**Fig. 2D**).

NIH or atherosclerotic de novo lesion was composed of SMC, inflammatory cells, red blood cells, amorphous materials, fibrin, and collagen in previous human studies^{6, 7, 9}.

When vascular SMC are exposed to a high glucose level, the response is proliferative and migratory, contributing to the progressive atherosclerosis and restenosis^{16, 17}. In our results in **Fig. 3** and **4B**, hyperglycemia increased macrophage infiltration and the non-SMC ratio in the NIH area without a marked increase of SMC. Cellular composition of plaque might not only influence plaque growth but also its stability. Previous reports in experimental and human atherosclerotic lesions suggest that increased macrophage activities mostly associated with inflammatory processes, extracellular lipid accumulation, or decreased SMC density might be the pathogenic basis for plaque instability^{18, 19}. Macrophages in the vascular wall can be selectively cleared via the induction of autophages by m-TOR inhibition²⁰. Stent-based delivery of the rapamycin derivative everolimus in ath-

erosclerotic plaques from cholesterol-fed rabbits led to a marked reduction in macrophage content via autophagic cell death without altering the amount of SMC^{20, 21}. In addition, monocytes from humans with poorly controlled type 1 or 2 DM exhibited increased adherence to the endothelial monolayer²², suggesting that a combination of increased endothelial adhesion molecule expression and increased ability of monocytes to adhere to these adhesion molecules contributed to the increased lesion initiation.

Vascular endothelial cells were injured also by high glucose condition. Experimentally induced hyperglycemia and hyperinsulinemia decreased arterial vasodilation in healthy individuals^{23, 24} by increasing superoxide generation and subsequent decreasing endothelial nitric oxide availability^{24, 25}. Endothelial alterations led to increased production of tissue factor²⁶, a strong pro-coagulant, and alterations in soluble coagulation and fibrinolytic factors. The results of our study well matched previous reports that DES implantation in SD rats with hyperglycemia increased VCAM-1 and VEGF expression. A study of a small group of human subjects with type 1 or type 2 DM showed increased endothelial VCAM-1 immunoreactivity compared with non-diabetic controls. Generally, up-regulated VCAM-1 increased the arrests of monocytes and lymphocytes while decreasing the adhesion of neutrophils²⁷. In patients with type 1 DM, endothelial expression of VEGF was significantly greater than in patients with type 2 DM and controls. The expression of VEGF plays a primary role in promoting the extravasation of inflammatory cells. On the other hand, VEGF was implicated in playing an important role in the mobilization of endothelial progenitor cells from bone marrow and homing of these cells to sites of vascular injury^{28, 29}.

In our results in **Fig. 5B**, hyperglycemia exhibited increased expressions of VCAM and VEGF (data not shown) at the endothelial monolayer and after DES implantation, and these over-expressions were sustained; however, in non-DM rats, DES implantation did not change the expression of VCAM. In addition, VEGF over-expression was stronger in the PES group; however, in NDM rats, DES implantation did not change the expression of VEGF.

In the present study, as seen in **Fig. 6A**, immature endothelial cells, c-kit-positive cells were defined as brown-stained cells at the NIH in DM rats with PES and NDM rats with SES. Both hematopoietic stem cells and endothelial progenitor cells fully express the stem cell factor receptor, c-kit; however, during the differentiation of endothelial progenitor cells into mature endothelial cells, c-kit expression is lost in

conjunction with the de novo appearance of the mature endothelial cell markers of vWF and VE-cadherin^{30, 31)}. In **Fig. 6B**, mature endothelial cells were defined as vWF-positive cells at NIH in NDM rats with SES and PES. Mature endothelial cells were located in the interspace between stent struts except in the upper portion.

Increased hyaluronan has been observed in the vasculature of diabetic patients³²⁾ and there is a significant correlation between the aortic content of hyaluronan and diabetes. The functions of hyaluronan in arterial tissue are not known, but increased hyaluronan accumulation in arterial lesions of atherosclerosis³³⁾ and mechanical arterial injury³⁴⁾ has been reported in humans. In addition, Baynes and colleagues demonstrated that reactive carbonyls, which are a feature of the diabetic state³⁵⁾, could fragment into hyaluronan³⁶⁾; however, the specific mechanisms by which diabetes stimulates hyaluronan accumulation are still not known. In our study, hyaluronan accumulation at the NIH only could be detected in NDM rats; however, hyaluronan content of media did not show a difference between NDM and DM rats.

To the best of our knowledge, this study represents the first animal experiments of SES and PES implantation under similar conditions of PCI in patients with type 1 DM. In humans, histological analysis at stented sites is restricted except at autopsy; therefore, the cellular and molecular mechanisms whereby diabetes accelerates cardiovascular disease and atherosclerosis are still poorly understood. In our study, with DES implantation, hyperglycemia induced more restenosis than in the control group. Restenosis in hyperglycemia was caused by both initial thrombus and inflammatory cell accumulation and, therefore, reendothelialization was delayed. These results provide a reliable pathological clue that hyperglycemia is a risk factor for restenosis of DES implantation and a therapeutic clue for using DES and the application of combined therapy using anti-thrombotic and anti-inflammatory drugs in diabetic patients.

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