Original Article

The effects of bare metal versus drug-eluting stent implantation on circulating endothelial cells following percutaneous coronary intervention[*]

Seyed Mohammad Hashemi Jazi[a], Saeed Shafiei[b],
Seyed Hamid Zarkesh-Esfahani[c], Saman Maleki Vareki[c],
Shaghayegh Haghjooy Javanmard[b]

Abstract

BACKGROUND: The purpose of this study was to compare the effects of bare metal stents (BMS) and drug-eluting stents (DES) implantation on circulating endothelial cells (CECs) which have been proposed as cellular markers of endothelial dysfunction following percutaneous coronary intervention (PCI). Recently, it has been established that DES further reduce restenosis and revascularization rate compared to bare metal stents in elective procedures. However, its benefits are compromised by the stent-related thrombosis events.

METHODS: 22 patients who were candidate of PCI were included in this study. The patients underwent DES implantation (n = 11) or BMS implantation (n = 11). In all patients the numbers of CECs were determined before and a week after stent implantation using flow cytometry and the obtained data were compared within and between groups by paired and unpaired Student's t-test, respectively. CECs were defined as cells negative for CD45 (FITC) and highly double positive for CD146 (PE) and CD34 (PE-Cy5) expression.

RESULTS: There were no significant differences in the baseline levels of CECs between two groups (p = 0.96). Stent implantation led to a significant increase in CECs compared with the preprocedural levels in the BMS group (p = 0.005) whereas there was a significant decrease in CEC numbers in DES group (p < 0.001). One week after stent implantation CECs count in BMS group was significantly higher compared to DES group (p < 0.001).

CONCLUSIONS: The results indicate that patients undergoing DES implantation were subjected to less endothelial injury than patients receiving BMS as indicated by CEC enumeration.

KEYWORDS: Angioplasty, Balloon, Coronary, Drug-Eluting Stents, Endothelial Cells.

Drug-eluting stents (DES) have become the treatment of choice for patients with symptomatic coronary artery disease undergoing percutaneous coronary revascularization.[1,2] Although these stents have reduced rates of restenosis compared to bare-metal stents (BMS), the late thrombosis, a life-threatening complication of these stents, has perceived as a major concern.[1,2]

The integrity and functional activity of the endothelial monolayer play a crucial role in the prevention of thrombosis.[3] The endothelium undergoes a constant process of injury and repair in response to mechanical injury of stenting.[4] Furthermore, it has been revealed that DES may cause much more endothelial dysfunction

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* This paper derived from a Specialty thesis in Isfahan University of Medical Sciences.
[a] Applied Physiology Research Center, Department of Physiology and Department of Cardiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
[b] Applied Physiology Research Center, Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran.
[c] Applied Physiology Research Center, Department of Physiology and Department of Immunology, School of Medicine, Isfahan University of Medical Sciences and Department of Biology, School of Sciences, The University of Isfahan, Isfahan, Iran.
[d] Department of Biology, School of Sciences, The University of Isfahan, Isfahan, Iran.
Corresponding Author: Shaghayegh Haghjooy Javanmard
E-mail: sh_haghjoo@med.mui.ac.ir

in coronary bed compared to that of BMS.[5,6] It has been shown that sirolimus reduces the replication of human endothelial cells.[7] Sirolimus also attenuates the recruitment of leukocytes and progenitor cells after vascular injury[8] and decreases production of vascular endothelial growth factor which is considered as a major and essential stimulus for vascular repair.[9] However, data regarding the extent of DES induced endothelial damage are lacking.

Endothelial damage is characterized by the detachment of endothelial cells from the sub endothelial matrix.[10] The enumeration of circulating endothelial cells (CECs) released in peripheral blood after vascular injury represents a direct investigation of the endothelium status.[10] CECs count is evolved as a novel method of assessing endothelial dysfunction.[11] Rarely found in the peripheral blood of healthy persons, CECs increase in a wide variety of diseases such as cardiovascular disease, cancer, infection and inflammatory states.[11] Increased CECs count in peripheral blood has been reported in various conditions with vascular injury such as stable angina and acute coronary syndromes and was strongly associated with the extent of endothelial injury.[10] Furthermore, increased CECs count served as an independent predictor of either death or major coronary adverse event at 30 days and 1 year.[10]

Since impaired endothelial recovery makes the vessel prone to acute thrombosis, the current strategies to lower the incidence of instent restenosis following percutaneous coronary intervention (PCI) are aimed to decrease the endothelial injury and increase arterial healing after stent injury. In addition, early restoration of endothelial integrity inhibits neointimal growth and thrombosis. So, it is necessary to find the approaches which encourage more endothelial recovery after stent injury.

The present work was undertaken to compare the effects of bare metal and drug-eluting stent implantation on circulating endothelial cells following PCI, as assessed by CEC numbers as a marker of endothelial dysfunction and damage before and one week after stent implantation.

Methods

Patient Population

22 patients with history of cardiovascular disease (CVD) who were candidate for elective coronary angioplasty and stent implantation were admitted in our study by convenient sampling method and underwent PCI in Shahid Chamran Hospital between January 2007 and February 2008. All the patients underwent coronary angiography and were diagnosed with significant coronary stenosis (> 75% cross sectional area).[12] The patients who had a de novo target lesion that was suitable for implanting (stent diameter ≥ 3 mm) either a BMS or a DES according to the vessel diameter, received a successful BMS (n = 11) or DES (n = 11) implantation by the judgment of the cardiologist. In the DES group, 3 patients were treated by sirolimus-eluting (Cypher) stents, but other 8 patients were treated by paclitaxel-eluting (Taxus) stents. In all cases, the culprit coronary artery was successfully recanalized. In each patient, CECs count, were determined before stenting and one week after PCI.

The exclusion criteria were having recent acute myocardial infarction (AMI) during two weeks before PCI, AMI during PCI and AMI at least one week after PCI, history of severe renal, hepatic disease, hematological disorders, acute or chronic inflammatory disease, malignancy, cardiogenic shock, and use of intra aortic balloon pump in patients. The study protocol was approved by the ethics committee of Isfahan University of Medical Sciences and before enrolment, written informed consent was obtained from all patients. All the technical staffs were blinded to study groups.

Determination of the Circulating Endothelial Cell Count

All the blood used in the analysis was freshly drawn and proceeded immediately. For determination of CECs counts, venous blood was drawn from the cubital vein. The first 7 ml were discarded and CECs were counted using flow cytometry technique. The general protocol for flow cytometry was obtained from Goon et al[13] with minor changes. Briefly, 1 ml

of EDTA treated blood was put in each 15 ml Falcon tube and the RBCs were lysed using 10 ml of FACS lysing solution (Becton Dickinson, 10 x, diluted 1:10) according to the manufacturer's instructions. Cells were washed with cell buffer solution (PBS + Bovine serum albumin (BSA) 1% + sodium azaide 0.05%) and centrifuged at 500 x g to replete the cells. The white cells were then blocked with 20 µl of human immunoglobulin (Octagam, Octapharma, Switzerland) and 200 µl of mouse serum (Sigma, Gillingham, UK) for a minimum of 20 min at room temperature. Then, the samples were incubated with fluorochrome-labelled monoclonal anti-human mouse antibodies, FITC-CD45, PE-CD146 and PE-Cy5-CD34 (Becton Dickinson, Oxford, UK) for 30 min in dark place at room temperature. The cells were washed, repelleted and made up to a final volume of 1 ml with cell buffer solution and analyzed immediately. Each sample was analyzed in a PAS/Dako flow cytometer (Partec, Denmark) with the use of acquisition/analysis programme FloMax 2.4 (Partec). Cells were plotted according to forward scatter and side scatter profiles (a measure of size and granularity of an event, respectively) and gated to include only mononuclear cell events and excluding cell doublets, platelets, dead cells/debris, microparticles and high side scatters events. A second gate was used to include only those cells negative for CD45 (FITC) low to medium side scatter singlets. A third gate was used to analyze cells doubly positive for CD146 (PE) and CD34 (PE-Cy5) expression and only high intensity doubly fluorescent cells were defined as CECs (Figure 1). Each sample was analyzed for a minimum of one million mononuclear cellular events. Flourochrome-matched isotype controls (FITC-IgG1, PE-IgG1, PE-Cy5-IgG1, Becton Dickinson) as well as non-stained samples were used to set the appropriate gate parameter and served as negative controls.

Statistical Analysis

The data are reported as mean ± standard error of the mean (SEM) and as percentages for categorical variables. The statistical software package, SPSS version 13.0 (SPSS Inc, Chicago, IL) was used to perform statistical analysis. The data were tested for normality and homogeneity of variance. Otherwise, paired Student's t-test was used to assess the significance of any change within groups, while unpaired Student's t-test was used to compare continuous variables between groups. Fisher's exact test was performed to compare categorical variables. A multiple regression analysis was carried out with CECs count as dependent variables and age, sex, hypertension, diabetes mellitus, hyperlipidemia, and smoking as independent variables. P value < 0.05 was considered statistically significant.

Results

Baseline Characteristics

Baseline clinical characteristics (Table 1) of

Table 1. Baseline characteristic of the patients

	DES*	BMS**	P value
Age (years)	53.6 ± 12.01	60 ± 8.67	0.99
Male (%)	50	71.43	0.07
Body mass index (kg/m^2)	25.3 ± 2.8	22.6 ± 3.8	0.02
Medical history	-	-	-
Myocardial infarction (%)	85.7	85.7	0.35
Hypertension (%)	78.6	64.3	0.16
Diabetes mellitus (%)	35.7	14.3	0.09
Hyperlipidemia (%)	78.6	67.9	0.10
Current smokers (%)	21.6	14.3	0.18
Systolic blood pressure (mmHg)	122 ± 13.8	132 ± 16.8	0.5
Diastolic blood pressure (mmHg)	80 ± 5.3	83 ± 4.5	0.09

Data are presented as mean ± SEM or % and analyzed by Student's t test or Fisher's exact test as appropriate.
* DES: Drug-Eluting Stents; ** BMS: Bare Metal Stents

both groups were similar, although the DES group had a significantly higher body mass index (BMI) than BMS group (p = 0.02).
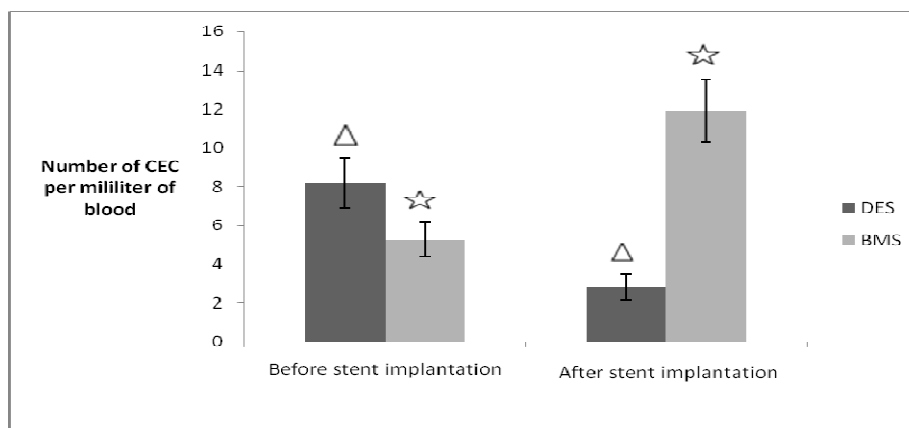
The Effect of Stent Implantation on CECs Count

The result of CECs enumeration in two groups of the patients has been shown in figure 2. There were no significant differences in baseline levels of CECs between the two groups (p = 0.96). Stent implantation led to a significant increase in the number of CECs compared with preprocedural levels in BMS group (p = 0.005) while a significant decrease (before vs. after) in number of CECs was observed in DES group (p < 0.001). One week after stent implantation, CECs count in BMS group was significantly higher compared to DES group (p < 0.001). None of the age, sex, hypertension, diabetes mellitus, hyperlipidemia, and smoking was the confounding factor on the difference of the significant difference between groups.
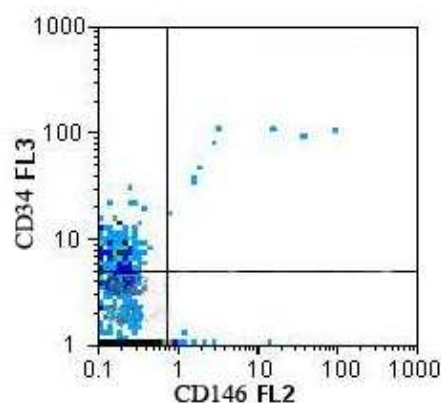
Figure 1. Three-colour flow cytometric analysis for circulating endothelial cells
Blood cells were plotted according to forward scatter and side scatter profiles and gated to include only mononuclear cells. A second gate was used to exclude CD45 positive cells. Cells doubly positive for high intensity of CD146 (PE) and CD34 (PE-Cy 5) expression (highlighted for clarity) were counted as CECs.

Figure 2. The Effect of Stent Implantation on CECs Count
There were no significant differences in baseline levels of CECs between the two groups (p = 0.96). Stent implantation led to a significant decrease in number of CECs in DES group and a significant increase in number of CECs compared with preprocedural levels in BMS group. One week after stent implantation CEC count in BMS group was significantly higher compared to DES group (p < 0.001).

△ (p < 0.001); ☆ p = 0.005

Discussion

The result of the current study showed that despite the similar count of CECs at the baseline; stent implantation led to a significant more CECs in the BMS group compare to DES group. These results remained unchanged after one week of stent implantation.

Using DES implantation is an advantageous therapeutic approach in patients with stable coronary artery disease or acute coronary syndrome.[14] However, there are only few publications available concerning the endothelial effects of DES. Previous works have been shown that stenting leads to significant increase in CECs counts compared with preprocedural levels.[9,15] This is in agreement with our results in BMS group as the patients had increases in their CECs counts compared with preprocedural levels.

Although, it has been revealed that DES may cause much more endothelial dysfunction in coronary bed compared to that of BMS,[5,6] the number of CECs was significantly lesser in DES group. It means that there was lower endothelial injury in this group. The underlying mechanism responsible for the decrease in CECs after PCI with DES is unclear. Mechanisms of endothelial detachment are also poorly understood.[16,17] In inflammatory states, various factors such as direct leukocytes attacks, cytokines, proteases and matrix metalloproteinase may play some key roles.[16,17] Therefore, the decrease in the number of CECs in DES group could be the result of local anti-inflammatory effects of sirolimus and paclitaxel.[18] It has been reported that patients undergoing DES implantation achieved more reductions in preprocedural markers of inflammation and necrosis than patients receiving bare metal stents among those with non-ST-elevation acute coronary syndrome.[18,19]

Several reports in the literature demonstrated the presence of coronary endothelial dysfunction related to DES when compared with BMS implantation.[20-22] However, there are some convincing evidences that the treatment of coronary stenosis with DES is highly effective and associated with a sustained clinical benefit up to 3 years after device implantation.[23]

A limitation of the present study was that the stent type was not randomized, and the number of enrolled patients was small. The data obtained from this study are preliminary, and large randomized studies are required in order to confirm the present results.

Conclusions

Although CECs have been accepted as a promising marker of active endothelial injury, its role in a clinical scenario remains to be evident. If we assume decreased CECs count as decreased endothelial injury and dysfunction, it can be concluded that DES not only prevent restenosis, but also protect endothelial function at least one week after stent implantation. So, less endothelial injury and more restoration of endothelial integrity which inhibit neointimal growth, vascular smooth muscle cells proliferation and thrombosis, may be the underlying mechanisms of lower incidence of restenosis with DES.

Acknowledgements

Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

SMHJ and SS did the patients admission, angiography and had contributions to conception and design of the study. SHZE and SMV did the flow cytometry, analysis and interpretation of data. SHJ had substantial contributions to conception and design of the study, analysis of the data and drafting the manuscript. All authors have read and approved the content of the manuscript.

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