CAPTURING ENDOTHELIAL CELLS BY CORONARY STENTS - FROM HISTOLOGY TO CLINICAL OUTCOMES

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HVATANJE ENDOTELNIH ČELIJA KORONARNIM STENTOVIMA - OD HISTOLOGIJE DO KLINIČKIH ISHODA

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ABSTRACT

Introduction of drug-eluting stents (DES) in the therapy of patients with coronary artery disease resulted in the significant reduction of in-stent restenosis compared to bare-metal stent (BMS) treatment. However, the high incidence of late stent thrombosis with DES emerged as one of the safety concerns after their implantation. Enhancing stent endothelialization by improved early healing and neointimal strut coverage emerged as possible solution for this late complication. Endothelial progenitor cells (EPC) capturing stents are designed to promote in situ endothelialization with immobilized, antihuman, anti-CD34 antibodies attached to the luminal stent surface. Anti-CD34 antibodies target and capture EPC from circulation, which further differentiate into vascular endothelial cells and form functional endothelial layer on the stent surface. These cells are also capable of secreting pro-angiogenic factors that stimulate local endothelial cells to proliferate and migrate. Preclinical and clinical studies proved feasibility, efficacy and safety of EPC capturing stents in stable and high-risk patients with coronary artery disease. Rapid and extensive endothelialization of EPC capturing stents translated into favorable profile of clinical outcomes, comparable to efficacy of BMSs and DESs. Therefore, we here present the most important results from the experimental and clinical studies that explored EPC capturing strategy to enhance endothelialization, reduce the incidence of in-stent thrombosis and improve outcomes of patients with coronary artery disease, along with the future perspectives in this promising therapeutic approach.

Keywords: coronary stent, endothelial progenitor cells, drug-eluting stent, bare metal stent, coronary artery disease

SAŽETAK

Primena stentova sa oslobađanjem leka (DES) u terapiji pacijenata sa koronarnom bolešću srca dovela je značajnog smanjenja pojave in-stent restenoze u poređenju sa primenom običnih metalnih stentova (BMS). Međutim, uočena je veća pojava kasne tromboze stenta nakon implantacije DES-a. Rešenje za ovu kasnu komplikaciju bi moglo biti povećanje endotelizacije stenta putem poboljšanja ranog neointimalnog pokrivanja stenta. Stentovi koji „hvataju“ endotelne progenitorske ćelije (EPC) kreirani su sa ciljem da poboljšaju in situ endotelizaciju pomoću imobilisanih, antihumanih, anti-CD34 antitela koja su pričvršćena za luminalnu stranu stenta. Anti-CD34 antitela su usmerena na EPC koje potom hvataju iz cirkulacije, a EPC se dalje diferenciraju u smeru vaskularnih endotelnih ćelija i formiraju funkcionalni sloj endotela na površini stenta. Ove ćelije imaju i sposobnost izlučivanja pro-angiogenih faktora koji stimulišu proliferaciju i migraciju okolnih endotelnih ćelija. Prekliničke i kliničke studije su dokazale efikasnost i bezbednost primene stentova koji „hvataju“ EPC kod stabilnih pacijenata sa koronarnom bolešću srca, ali u visoko-rizičnoj grupi pacijenata. Pokazalo se da brza i obimna endotelizacija stentova koji „hvataju“ EPC daje povoljne kliničke ishode koji se mogu porediti sa ishodima nakon ugradnje BMS i DES. Stoga je cilj ovog rada da predstavimo najvažnije rezultate eksperimentalnih i kliničkih studija koje su ispitivale mogućnost „hvatanja“ EPC u cilju poboljšanja endotelizacije, smanjenja učestalosti in-stent tromboze i poboljšanja ishoda pacijenata sa koronarnom bolešću srca, kao i moguće sledeće korake u ovom obećavajućem terapijskom pristupu.

Ključne reči: rana neonatalna sepsa, dijagnoza, terapija

ABBREVIATIONS

BMS – bare metal stent, **DES** – drug-eluting stent, **EPC** – endothelial progenitor cell, **PCI** – percutaneous coronary intervention, **LST** – late stent thrombosis, **SES** – sirolimus-eluting stent, **OCT** – optical coherence tomography, **SEM** – scanning electron microscopy, **MACE** – major adverse cardiovascular event, **QCA** – ..., **IVUC**, **STEMI**, **TIMI**...etc.

INTRODUCTION

Percutaneous coronary intervention (PCI) has become the treatment of choice for the majority of patients with coronary artery disease. Despite the efficacy of PCI, with the appearance of bare metal stents (BMS), the rate of in-stent restenosis was reaching up to 30% of cases (1, 2). Immunologic response to the presence of metal stent within coronary circulation, as well as mechanical injury of the vessel wall by the stent deployment, often resulted in neointimal hyperplasia. Vascular smooth muscle cell proliferation, along with the deposition of extracellular matrix, can lead to progressive narrowing and occlusion of blood vessel, so-called in-stent restenosis (3). The introduction of drug-eluting stents (DES) with their antiproliferative properties significantly reduced the growth of neointima and the restenosis rate (4, 5). Yet, the consequence of cytotoxic/cytostatic effects of the DES appeared to affect the endothelial cells as well, inhibiting their proliferation, migration and function in the long-term (6, 7). This resulted in the occurrence of the late stent thrombosis (LST), one of the safety concerns following the DES implantation (8, 9).

Late stent thrombosis in DES is histologically characterized by fibrin deposition, scant presence of smooth muscle cells and incomplete endothelization. Finn et al. (10) proposed that the delayed endothelization is one of the main causes of the late stent thrombosis. Additional proposed mechanism of the late stent thrombosis included hypersensitivity reaction (11), excessive fibrin deposition with malapposition, or neoatherosclerosis (12).

Therefore, enhancing endothelization emerged as an interesting approach in attenuating antiproliferative effects of DES and overcoming the risk of late stent thrombosis. Capturing circulating endothelial cells could be a promising technique, especially in high-risk patients unable to receive dual antiplatelet therapy (DAPT) after stent implantation for longer periods of time.

ENDOTHELIAL PROGENITOR CELLS

Endothelial progenitor cells (EPC) have first been isolated and described by Asahara et al. (13). These circulating cells derive from the bone marrow, differentiating from their ancestor cell hemangioblast, and express surface cell markers in similar pattern as vascular endothelial cells. EPC are capable of migrating to ischemic or hypoxic regions, where they can adhere to injured endothelium and promote angiogenesis or regeneration of the damaged endothelium (14). Since there is no specific and distinctive marker for EPC, the panel of surface cell markers is usually used for their identification. Traditionally, co-expression of CD34/VEGFR2(KDR)/CD133 surface markers is used to identify human circulating EPCs (13). However, more “mature” forms of EPC progressively lose CD133 expression, which leave more committed EPCs with CD34+VEGFR2+ phenotype.

EPC CAPTURING STENTS – DEVELOPMENT

Among many antibody-targeted surface markers of circulating EPC potentially capable of capturing these cells from circulation, CD34 has been shown to be the most specific molecule in this setting (15). CD34 is a transmembrane glycoprotein expressed on the cell surface, important in regulation of cells’ proliferation and differentiation (16). It is traditionally described as hematopoietic stem cells marker, but its expression is proven on several non-hematopoietic progenitor lineages, such as vascular endothelial progenitors (17), epithelial progenitors (18) and embryonic fibroblasts (19). CD34 is considered to be “pan-endothelial” marker, expressed on early and mature vascular endothelial cells. Along with CD133 and VEGFR2, CD34 counts into crucial markers for identification of EPCs (13).

EPC capturing stents are designed in such a manner that immobilized, monoclonal, antihuman CD34 antibodies are attached to the luminal surface of the stent, where they target and capture CD34 immunopositive (CD34⁺) circulating cells. Initially, it was hypothesized that the “captured” EPCs incorporate into the vessel wall, and further differentiate into new endothelial cells. Additional mechanism was proposed, where the recruited EPCs adhered to denuded endothelium, secreted pro-angiogenic factors that stimulated resident endothelial cell to proliferate and migrate in order to form intimal layer (20, 21).

The first stent coated with anti-CD34 antibodies was tested in 2003 on experimental murine model (22). This novel therapeutic strategy resulted in rapid endothelization of implanted stents, while adherent CD34⁺ cells were present on the stent surface after 1 hour of incubation. Experimental studies that followed aimed at comparing EPC capturing stents to BMS/DES.

The comparison of EPC capturing stent with BMS in the animal model also showed positive results for EPC strategy (23). Early endothelization on the 2nd day was significantly higher with anti-CD34 antibody covered stents compared to BMS (68% vs. 53%), although the endothelization rate was similar on the 5th day (above 95%). Additionally, neointimal thickness did not show difference between the two stent groups on 28th and 90th day. Greater rate of endothelization by EPC capturing stent vs. BMS on the 7th day was also confirmed by scanning electron microscope (SEM) and quantitative PCR in rabbit aorta and ilio-femoral injury model (24).

Nakazawa et al. (25) assumed that the implantation of EPC capturing stent (Genous stent Technology, OrbusNeich Medical, Fort Lauderdale, Florida) could intensify endothelization of sirolimus-eluting stent (SES) compared to SES alone. That hypothesis was tested on porcine coronary arteries where endothelization was confirmed by CD31 expression (another well-known endothelial cell marker) using confocal microscopy and SEM. Fourteen days after single stent implantation, the highest percentage of stent endothelization was recorded in EPC capturing stent (99%), the endothelial

cells' coverage of overlapping segments (SES with EPC capturing stent) was 79%, while the endothelialization percentage was the lowest in SES alone (55%). Examining different stent overlapping combinations, authors noted that the endothelialization of overlapping segment was the highest with EPC + EPC stent (95%), lower, but still significantly high, in EPC + SES (79%) compared to overlapping segment of SES + SES (36%). These results confirmed greater re-endothelialization success of EPC capturing stents, regardless of overlapping with SES. Granada et al. (26) confirmed these results on a swine model, observing greater endothelial maturation on EPC capturing stent than on SES by quantitative CD31 expression with confocal microscopy. Additionally, optical coherence tomography (OCT) and histological analysis showed no difference in neointimal thickness between EPC capturing stent, SES and everolimus-eluting stent (EES) on the 28th day.

Tan et al. (27) examined the novel nanocomposite polymer surface (POSS-PCU) as a platform for sequestration of EPC via immobilized anti-CD34 antibodies. The number of EPC colonies was assessed by fluorescence microscopy and SEM, showing no difference between anti-CD34-coated POSS-PCU surface and POSS-PCU surface with no antibodies attached. On the other hand, the presence of antibodies significantly improved hemocompatibility, which resulted in prolonged clotting times, decreased platelet adhesion and activation. These results pointed to the importance of the stent surface and adequate surface-antibody combination to attract, capture EPCs and promote endothelialization.

EPC CAPTURING STENTS – CLINICAL TRIALS

Data from first-in-man clinical trial with EPC capturing stent (Genous®) were published in 2005 (28). In all 16 enrolled patients with unstable angina or silent ischemia from HEALING-FIM registry complete angiographic and procedural success was accomplished. After 9-month follow-up, major adverse cardiovascular events (MACE) were recorded in only one patient, related to symptom-driven target vessel revascularization, without any evidence of stent thrombosis. HEALING II, a nonrandomized clinical registry study, included more patients with stable coronary artery disease (n=63) with quantitative coronary angiography (QCA) and intravascular ultrasound (IVUS) follow-up after 6 and 18 months (29). Significant late regression of neointimal hyperplasia was recorded by QCA between 6 and 18 months of follow-up (24,4% reduction) as well as with IVUS (11,4% reduction). Reduction in neointimal hyperplasia correlated with the number of circulating EPC, where patients with lower EPC titers at 6-month follow-up had greater late lumen loss compared with patients with normal EPC titers. This finding was suggestive of EPC-mediated vascular repair response.

Since the authors of HEALING II study observed beneficial effects of statin therapy on the number of circulating EPC and improved angiographic outcomes, HEALING IIB study

tested the efficacy and safety of Genous stent in conjunction with the optimal statin therapy to induce and maximize EPC recruitment (30). Despite statin therapy, results in late lumen loss were similar to HEALING II data. However, statin therapy indeed increased the number of circulating EPCs by 5.6-fold. The same year, data from the largest Genous stent eHEALING registry were published with 4939 stable patients included to obtain the “real world” data (31). After 12-month follow-up, the rate of target vessel failure (defined as target vessel-related cardiac death or myocardial infarction, and target vessel revascularization) was 8.4%, while the incidences of target lesion revascularization and stent thrombosis were low (5.7% and 1.1%, respectively).

Wohrle et al. (32) tested whether combination of paclitaxel-coated balloon with EPC capturing stent could improve endothelialization and reduce neointimal proliferation in 120 patients with native coronary arteries. Treatment with paclitaxel-coated balloon with EPC stent was superior to EPC alone in terms of in-stent late lumen loss after 6 months. Additionally, clinical events (composite of cardiac death, myocardial infarction due to target vessel or target lesion revascularization) were reduced from 17.2% to 4.8%, with no stent thrombosis recorded in both groups.

EPC capturing stents proved their feasibility and safety in patients with ST-elevation acute myocardial infarction (STEMI) without cardiogenic shock during primary PCI (33). This clinical study enrolled 120 STEMI patients, where the majority of them (95%) achieved Thrombolysis in Myocardial Infarction (TIMI) flow 3 after PCI. MACE rate was 1.6% at the hospital, 4.2% after 30 days, 5.8% after 6 months, and 9.2% after 1 year of follow-up. Two patients experienced acute and subacute stent thrombosis, with no occurrence of late stent thrombosis.

Choi et al. (34) reported significantly improved microvascular dysfunction 6 months after the implantation of EPC capturing stent compared to DES (zotarolimus- or everolimus-eluting stent). Although coronary flow reserve (CFR) was not significantly different among different stent groups after 6-month follow-up, patients with EPC stent had significantly lower index of microvascular resistance (IMR) compared to patients with second-generation DESs.

Implantation of EPC capturing stent in patients with high risk of restenosis gave better long-term results than paclitaxel-eluting stent. After 1-year follow-up, target vessel failure was 17.3% in EPC stent group vs. 10.5% in paclitaxel-eluting group (35). However, 4 patients in paclitaxel-group had definitive stent thrombosis, with no stent thrombosis occurring in EPC stent-group. After 2-year follow-up, the difference in target vessel failure remained higher in EPC-group than in paclitaxel-group, although the difference was nonsignificant (20.4% vs. 15.8%, respectively) (36).

Chong et al. (37) reported the trend towards lower MACE rate with EPC capturing stent compared with sirolimus-eluting bioabsorbable polymer stent and BMS in patients with

acute myocardial infarction treated with primary PCI at 2-year follow-up (13.7% vs. 15.1% vs. 19.7%). The largest study with STEMI patients treated with EPC stent (n=321) reported cumulative MACE rate of 8.1% after 30 days, 10% after 6 months and 12.2% after 1-year follow-up (38). There was no evidence of late stent thrombosis, while one patient had acute stent thrombosis and two patients had subacute stent thrombosis.

On the other hand, HEALING registries initially observed that EPC capturing stent did not decrease late lumen loss to the same extent observed for DES (29, 39, 40). The HEALING IIB study, as well as eHEALING registry, confirmed these findings, with late lumen loss being higher with EPC capturing stents than observed for DES (30, 31), and also observed these results when comparing EPC capturing stents to newer DES generation (35, 41). These results initiated novel, modified EPC stent strategies.

Combining abluminal, bioabsorbable polymer with immobilized luminal anti-CD34 antibody along with drug-eluting stent properties emerged as an interesting therapeutic approach that could enhance *in situ* endothelialization and diminish the risk of late stent thrombosis. Well-designed Combo stent (*OrbusNeich, Hoevelaken, the Netherlands*) which merged bioabsorbable polymer, CD34 antibody coating and sirolimus as an eluting agent, initially showed less neointimal thickness with a higher degree of platelet and endothelial cell adhesion molecule expression compared to SES and EES in the animal model (42). Afterwards, Combo stent was compared to paclitaxel-eluting stent in 183 patients with single *de novo* native coronary artery lesion (43). This EPC capturing Combo stent proved non-inferior when analyzing in-stent late lumen loss after 9 months and MACE after 12-month follow-up. Additionally, no stent thrombosis was reported in either stent group. Results from the REMEDEE registry showed excellent results with Combo stent in routine clinical practice (44). One-year results of 1000 all-comers patients with implanted Combo stent showed the rate of target lesion failure of 5.7%, cardiac death of 1.7%, target vessel myocardial infarction of 0.7%, and target lesion revascularization of 4.4%. Promising results have also been reported with the Combo stent implantation in patients with acute coronary syndrome (45) and diabetic patients (46).

Recently published results from the HARMONEE (The Japan-United States of America Harmonized Assessment by Randomized Multicenter Study of OrbusNEich's Combo StEnt) registration study demonstrated the efficacy of Combo stent compared to Xience, contemporary DES (47). A total of 572 patients with ischemic coronary disease and non-ST elevation acute coronary syndrome were randomized to receive Combo stent or Xience stent. The primary endpoint was target vessel failure, with estimated value of 9% in the

Combo-group. However, target vessel failure was even lower than expected in patients receiving EPC-stent (7%) after 12 months vs. 4.2% in DES-group, meeting the non-inferiority study goal. Additionally, OCT after 12 months showed significantly better stent coverage with healthy tissue with Combo stent than with Xience (91.3 vs. 74.8), with more homogenous texture of neointima again with Combo stent (81.2% vs. 68.8%, respectively).

A lot is expected from the ongoing SORT OUT X trial that aims to compare efficacy and safety of Combo stent with contemporary, third-generation SES, with planned 3140 enrolled patients in a population-based setting, using registry detection of clinically driven events (48).

FUTURE PERSPECTIVE

Balancing thrombotic and hemorrhagic risk is essential in patients with stent implantation during PCI. Although DES significantly reduced the rate of in-stent restenosis compared to BMS, late stent thrombosis appeared as one of the late DES complications. One of the most significant predictors of late stent thrombosis is endothelial coverage. Therefore, improving endothelialization by EPC capturing stents is an intriguing approach that showed promising results in clinical trials with comparable efficacy to BMSs and DESs.

On the other hand, not all DESs with delayed stent healing exhibit late stent thrombosis (49, 50). This late complication can occur late after the cytostatic/cytotoxic drug has eluted from the stent. Another proposed mechanism for late stent thrombosis could be the chronic vascular irritation or endothelial dysfunction induced by stent, drug or coating (51, 52). Therefore, future investigation and trials might be focused on improving endothelial dysfunction, parallel to enhancing endothelialization.

Choosing a specific surface marker for EPC seems to be the greatest obstacle in this research field. Antibodies attached to the coronary stent should have high affinity, specificity and selectivity to capture EPCs, without binding to other blood cells. Since EPC are sparsely present in the blood flow, such ideal capture molecule should be able to promote EPC mobilization, or even their proliferation.

"Pro-healing" stent technology is a promising therapeutic approach that could significantly reduce the long-term risk of in-stent thrombosis by improving endothelialization. On the other hand, this technology could shorten or even eliminate the DAPT treatment and benefit high-risk patients.

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