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ORIGINAL ARTICLE

Interleukin-1β is associated with coronary endothelial dysfunction in patients with mTOR-inhibitor-eluting stent implantation

Hidetoshi Chibana[1] · Hidemi Kajimoto[1] · Takafumi Ueno[1] · Shinji Yokoyama[1] · Ken-ichiro Sasaki[1] · Masanori Ohtsuka[1] · Hiroshi Koiwaya[1] · Takaharu Nakayoshi[1] · Yoshiaki Mitsutake[1] · Naoki Itaya[1] · Masahiro Sasaki[1] · Yoshihiro Fukumoto[1]

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Abstract Implantation of mammalian target of rapamycin (mTOR)-inhibitor drug-eluting stents (DESs) impairs coronary endothelial function. There are no known non-invasive biomarkers of coronary endothelial dysfunction. We aimed to assess the association between serum interleukin-1beta (IL-1β) and coronary endothelial dysfunction in patients with mTOR-inhibitor DES implantation and to investigate the association between the mTOR pathway and IL-1β. We enrolled 35 patients who had implanted DESs for coronary artery disease. At a 10-month follow-up, peripheral venous blood samples were collected to measure IL-1β levels. Coronary endothelial dysfunction was evaluated by intracoronary infusion of incremental doses of acetylcholine. Serum IL-1β levels were significantly associated with the magnitude of vasoconstriction to acetylcholine at the segment distal ($P<0.05$) but not proximal to the stent. Serum IL-1β levels were positively correlated with stent length ($P<0.05$). To examine the direct effects of mTOR inhibition on IL-1β release, sirolimus was incubated in cultured human umbilical vein endothelial cells (HUVECs) or coronary artery smooth muscle cells (CASMCs). Sirolimus directly increased *IL-1β* mRNA expression ($P<0.01$) and enhanced IL-1β release into the culture media ($P<0.01$) in CASMCs, but not in HUVECs. Inhibition of mTOR triggers IL-1β release through transcriptional activation in CASMCs. Serum IL-1β levels are a potential biomarker for mTOR-inhibitor DES-associated coronary endothelial dysfunction.

Keywords Drug-eluting stent · Endothelial dysfunction · Biomarker · Coronary artery

Abbreviations

DES	Drug-eluting stent
IL-1β	Interleukin-1 beta
Ach	Acetylcholine
CASMCs	Coronary artery smooth muscle cells
BMS	Bare-metal stent
mTOR	Mammalian target of rapamycin
NTG	Nitroglycerin
HUVECs	Human umbilical vein endothelial cells

Introduction

The present generation of mammalian target of rapamycin (mTOR)-inhibitor drug-eluting stents (DESs) has dramatically reduced in-stent restenosis and target lesion revascularization rates compared with those in bare-metal stents (BMSs) after percutaneous coronary intervention. Within 1-year post-procedure, DES implantation results in a reduction in the target lesion revascularization rate compared with BMSs (DES: 0–4% vs. BMS: 17–23%) [1, 2]. However, long-term outcomes with DESs versus BMSs are inconsistent [3–6]. Norwegian Coronary Stent Trial (NORSTENT) has recently shown that rates of repeat revascularization were lower in the DES group, with no significant differences in the rates of death, myocardial infarction, and quality of life at 6 years of follow-up between the DES and BMS groups [5]. Possible interaction of the potent anti-proliferative agent and permanent non-biodegradable synthetic

✉ Hidemi Kajimoto
kajimoto@med.kurume-u.ac.jp; ozawh@hotmail.com; hkajimoto@cardiology.washington.edu

[1] Division of Cardio-Vascular Medicine, Department of Internal Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan

polymer has raised concerns regarding delayed arterial healing and poor re-endothelialisation at the stent site [7–9]. These may lead to impaired endothelial dysfunction at the segments adjacent to the site of DES implantation [10–12]. With regard to coronary endothelial dysfunction, abnormal vasoconstriction is shown by acetylcholine (Ach) infusion [10, 13–16], rapid atrial pacing [17], or exercise in coronary angiography [18]. However, currently, there is no useful non-invasive biomarker for detecting coronary endothelial dysfunction after DES implantation.

Proinflammatory cytokine interleukin-1beta (IL-1β) proteins are translated as 31-kDa precursors (pro-IL-1β) and are cleaved by caspase-1 into active "mature" 17-kDa forms through cleavage [19]. Pro-IL-1β transcription is induced by nuclear factor-kappa B activation [20]. Healthy endothelium generates nitric oxide (NO), which maintains vascular homeostasis and normal vasomotor tone. However, in pathophysiological situations, excess generation of IL-1β may decrease NO bioactivity and bioavailability [21–23]. IL-1β decreases endothelial NO synthase gene expression through inhibition of p38 phosphorylation [24, 25]. Therefore, we speculate that elevated serum IL-1β levels are a potential biomarker of endothelial dysfunction in patients with DES implantation. The present study aimed to examine the association between serum IL-1β levels and coronary endothelial dysfunction in patients with mTOR-inhibitor DES implantation and to investigate the possible mechanism of mTOR-IL-1β signaling pathway.

Materials and methods

Study protocol

We enrolled 35 patients in this study who were diagnosed with coronary artery disease, including silent myocardial ischemia, stable angina, and restenosis of a BMS site, from April 2011 to June 2012. All mTOR-inhibitor DESs were implanted using the standard percutaneous coronary intervention techniques. Sirolimus-eluting stents (Cypher, Cordis Corporation, Miami Lakes, FL, USA) were implanted in two patients, a zotarolimus-eluting stent (Endeavor, Medtronic, Inc. Santa Rosa, CA, USA) in one, everolimus-eluting stents (Xience V or Prime, Abbott Vascular, Santa Clara, CA, USA; Promus Element, Boston Scientific, Natick, MA, USA) in 17, and biolimus-eluting stents (Nobori, Terumo, Tokyo, Japan) in 15. Patients with the following conditions were excluded from this study: acute coronary syndrome, angiographic in-DES restenosis; clinical or angiographic history of coronary vasospasm; severe chronic kidney disease (creatinine level >2.0 mg/dl); asthma; symptomatic congestive heart failure; and severe left ventricular dysfunction (left ventricular ejection fraction <30%). Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's review board.

Evaluation of coronary endothelial function

Coronary endothelial function was evaluated by measuring coronary vasomotion in response to Ach (Sigma–Aldrich, St Louis, MO, USA) at a 10-month follow-up. All vasoactive drugs, including calcium-channel blockers, long-acting nitrates, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and beta-blockers, were discontinued at least 24 h before the procedure. After baseline angiography, the endothelium-dependent vasomotor response was evaluated by intracoronary infusion of incremental doses of Ach at 10^{-8}, 10^{-7}, and 10^{-6} mol/l for 2 min. There was an interval of at least 3 min between each infusion. A temporary pacemaker was inserted through the femoral or brachial vein in all the patients. Thereafter, the endothelium-independent vasomotor response was tested by an intracoronary bolus infusion of 200 μg nitroglycerin (NTG; Eisai, Tokyo, Japan). Angiography was repeated 2 min after each drug infusion. The maximal vasomotor responses to Ach and NTG infusions were measured by quantitative coronary angiography with the CASS II system (Pie Medical BV, Maastricht, The Netherlands). Quantitative coronary analysis measurements were performed by an independent blinded observer. We evaluated vasomotor responses at the two segments, 5–15 mm proximal and distal to the stent, which were most constricted by Ach. We did not measure proximal coronary vasomotion in patients, whose stents were located in the ostial lesion of the coronary artery ($N=10$), because these precluded measurement of coronary vasomotion at the segments proximal to the stent site. In addition, as a reference, we evaluated an angiographically normal segment in another vessel. Changes in the vessel diameter in response to Ach and NTG infusions were calculated as the percentage of change versus baseline diameter. Endothelial dysfunction was described by the increased area under the curve (AUC) of cumulative Ach concentration-diameter changes at the segments proximal and distal to the stent site.

Measurement of serum IL-1β levels

Peripheral venous blood was drawn from the femoral or brachial sheath at the beginning of the procedure to avoid contamination with contrast fluid. Blood was allocated to different containers and centrifuged at 1500×*g* for 10 min. Serum was stored at −80 °C until use. Serum IL-1β levels were measured by a high-sensitivity ELISA (R&D Systems Inc., Minneapolis, MN, USA).

Cell culture

Human umbilical vein endothelial cells (HUVECs) and coronary artery smooth muscle cells (CASMCs) were purchased from Lonza (Basel, Switzerland). HUVECs (2×10^5 cells/ml) were cultured in EGM-2 medium (Lonza) supplemented with 2% fetal bovine serum. CASMCs (2×10^5 cells/ml) were cultured in SmGM medium (Lonza) supplemented with 5% fetal bovine serum. Passages 5–9 were used for the experiments. Cells were growth-arrested in serum-free medium for 24 h and then incubated with 10^{-6} mol/l sirolimus (rapamycin, Sigma–Aldrich) or vehicle for 12 h. The reaction was terminated by aspirating the medium. IL-1β release was estimated by measuring IL-1β levels in the conditioned medium with a high-sensitivity ELISA kit (R&D Systems Inc.). After three washes with ice-cold phosphate-buffered saline, cells were homogenized in Trizol (Life Technologies Corporation, Carlsbad, CA, USA).

Quantitative real-time PCR

RNA was isolated with an RNeasy Plus Mini Kit (Qiagen, Valencia, CA, USA). PCR primers for *IL-1β* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were purchased from Life Technologies Corporation. Real-time PCR was performed with cDNA samples using the TaqMan Gene Expression Master Mix (Life Technologies Corporation). Gene relative expression was calculated in relation to *GAPDH* quantitative expression and normalized.

Immunoblotting

For immunoblotting, we used monoclonal rabbit antibody to human caspase-1 and monoclonal rabbit antibody to human cleaved caspase-1 (Asp297) (both purchased from Cell Signaling Technology, Danvers, MA).

Cells were growth-arrested in serum-free medium for 24 h and then incubated with 10^{-6} mol/l sirolimus or vehicle for 20 h. The reaction was terminated by aspirating the medium. After three washes with ice-cold phosphate-buffered saline, cells were homogenized in lysis buffer containing a protease inhibitor cocktail using a FastPrep homogenizer (Thermo Savant, Holbrook, NY). Samples were then stored at −80 °C until use. Aliquots of cell lysate were separated on 4–12% sodium dodecyl sulphate–polyacrylamide gels by electrophoresis. Gels were then subjected to immunoblotting using a primary antibody (1:100 dilution), followed by a peroxidase-conjugated anti-rabbit secondary antibody (1:5000 dilution). Immunoreactive protein bands were detected using ECL western blotting reagents (Thermo Fisher Scientific, Waltham, MA). The intensity of immunoreactive protein bands was quantified by densitometry using the ImageJ software (National Institutes of Health, Bethesda, MA). Caspase-1 and cleaved caspase-1 were detected on the same gel following re-probing of the membranes.

Statistical analysis

Results are expressed as mean ± standard error. The AUC was calculated by the GraphPad Prism (GraphPad, San Diego, CA, USA) computer software using non-linear sigmoid curve fitting. The associations between IL-1β concentrations and stent length or the AUC were analysed by simple linear regression analysis for continuous variables and by the Mann–Whitney *U* test for categorical variables. Intergroup differences were assessed by the Mann–Whitney *U* test or one-way analysis of variance followed by Tukey–Kramer post-hoc analysis. A value of $P<0.05$ was considered statistically significant.

Results

Baseline and procedural characteristics

A total of 35 patients (67.9 ± 1.5 years) were enrolled in this study. Table 1 shows the baseline clinical and procedural characteristics. Thirty-one patients were treated by dual antiplatelet therapy (acetylsalicylic acid and clopidogrel). Vasoconstriction to Ach (AUC) was not correlated with baseline patients' characteristics or medications (Table 2).

Serum IL-1β levels and coronary endothelial function

At a 10-month follow-up, vasoconstriction to Ach was significantly greater at the segment distal to the stent than at the segment proximal to the stent ($P<0.01$, Fig. 1a, b). Endothelium-independent vasodilatation to NTG proximal and distal to the stent was comparable with that at the reference arteries (Fig. 1c). The mean serum IL-1β level was 0.226 ± 0.04 pg/ml (range 0–0.854 pg/ml). Simple linear regression analysis showed that serum IL-1β levels were not correlated with baseline patients' characteristics or medication, although they were positively correlated with stent length ($r=0.36$, $P<0.05$, Table 2; Fig. 2). There was also no correlation between serum IL-1β levels and C-reactive protein (data were not shown).

We divided the patients into two groups by the median value of serum IL-1β levels (0.152 pg/ml). We found that endothelium-dependent vasoconstriction was significantly more severe in the high IL-1β group ($n=18$) at the segments distal to the stents than in the low IL-1β group ($n=17$, $P<0.05$, Fig. 3a). At the segments proximal to the

Table 1 Baseline clinical and procedural characteristics

	Average level
Clinical characteristics	
Age (years)	67.9 ± 1.5
Male	25 (71.4%)
Body mass index (kg/m^2)	23.9 ± 0.5
Family history of CAD	6 (16.7%)
Hypertension	29 (82.8%)
Systolic blood pressure (mmHg)	122.8 ± 2.4
Diastolic blood pressure (mmHg)	73.4 ± 1.7
Diabetes mellitus	17 (48.6%)
Hemoglobin A1c (%)	6.3 ± 0.1
Smoking	14 (40%)
LDL-cholesterol (mg/dl)	89.8 ± 3.7
HDL-cholesterol (mg/dl)	52.1 ± 1.8
eGFR (ml/min/1.73 m^2)	69.5 ± 3.0
Pro BNP (pg/ml)	145.1 ± 33.5
Left ventricular ejection fraction (%)	67.3 ± 1.2
Post-PCI medications (%)	
ASA	34 (97.1%)
Clopidogrel	32 (91.4%)
Beta-blockers	15 (42.9%)
ARB or ACEI	23 (65.7%)
Calcium-channel blocker	10 (28.6%)
Nitrate	4 (11.4%)
Statins	25 (71.4%)
AHA/ACC type B2 or C	28 (80%)
Stent site	
Left anterior descending	22 (63%)
Left circumflex	7 (20%)
Right coronary artery	6 (17%)
Stent	
Length (mm)	32.3 ± 3.0
Diameter (mm)	3.1 ± 0.1
Deployment pressure (atm)	13.9 ± 0.6
TVR	0 (0%)

Values are mean ± SE or *n* (%)

LDL low-density lipoprotein, *HDL* high-density lipoprotein, *eGFR* estimated glomerular filtration rate, *BNP* brain natriuretic peptide, *ASA* acetylsalicylic acid, *ARB* angiotensin receptor blocker, *ACEI* angiotensin-converting enzyme inhibitor, *AHA* American Heart Association, *ACC* American College of Cardiology, *TVR* targeted vessel revascularization, *BES* biolimus-eluting stent, *EES* everolimus-eluting stent, *ZES* zotarolimus-eluting stent, *SES* sirolimus-eluting stent

Table 2 Simple linear regression analysis for determination of the area under the curve and serum IL-1β levels

	vs AUC		vs IL-1β	
	P value	*r*	*P* value	*r*
Clinical characteristics				
Age (years)	0.28	0.19	0.12	0.26
Male	0.92		0.86	
Body mass index (kg/m^2)	0.55	0.11	0.34	0.17
Family history of CAD	0.14		0.1	
Hypertension	0.73		0.44	
Systolic blood pressure (mmHg)	0.74	0.06	0.81	0.04
Diastolic blood pressure (mmHg)	0.91	0.02	0.53	0.11
Diabetes mellitus	0.1		0.26	
Hemoglobin A1c (%)	0.47	0.13	0.78	0.05
Smoking	0.99		0.55	
LDL-cholesterol (mg/dl)	0.66	0.08	0.24	0.2
HDL-cholesterol (mg/dl)	0.87	0.03	0.63	0.08
eGFR (ml/min/1.73 m^2)	0.32	0.17	0.08	0.3
pro BNP (pg/ml)	0.32	0.17	1	<0.01
Left ventricular ejection fraction (%)	0.24	0.2	0.35	0.16
post-PCI medications (%)				
ASA	0.24		0.08	
Clopidogrel	0.48		0.7	
beta-blockers	0.69		0.4	
ARB or ACEI	0.85		0.89	
Calcium-channel blocker	0.73		0.62	
Nitrate	0.34		0.9	
Statins	0.13		0.54	
AHA/ACC type B2 or C	0.74		0.93	
Stent site				
Left anterior descending	0.92		0.19	
Left circumflex	0.34		0.07	
Right coronary artery	0.38		0.79	
Stent				
Length (mm)	0.15	0.25	0.04	0.36
Diameter (mm)	0.6	0.09	0.97	<0.01
Deployment pressure (atm)	0.3	0.18	0.26	0.2
TVR	1		1	

LDL low-density lipoprotein, *HDL* high-density lipoprotein, *eGFR* estimated glomerular filtration rate, *BNP* brain natriuretic peptide, *ASA* acetylsalicylic acid, *ARB* angiotensin receptor blocker, *ACEI* angiotensin-converting enzyme inhibitor, *AHA* American Heart Association, *ACC* American College of Cardiology, *TVR* targeted vessel revascularization, *BES* biolimus-eluting stent, *EES* everolimus-eluting stent, *ZES* zotarolimus-eluting stent, *SES* sirolimus-eluting stent

stents, endothelium-dependent vasoconstriction was not observed in the high IL-1β ($n=13$) and low IL-1β groups ($n=12$, Fig. 3b). These results were confirmed by AUC analysis (Fig. 3c, d). By simple linear regression analysis, the AUC was positively correlated with serum IL-1β levels at the segments distal to the stents ($r=0.38$, $P<0.05$, Fig. 3c), but not at the segments proximal to the stents (Fig. 3d). Table 3 shows AUC and serum IL-1β levels at the segments distal to the different types of drug-eluting stents. It seems that patients with sirolimus-eluting stents might have poorer endothelial function and higher IL-1β levels than others (Table 3). This finding suggested that coronary

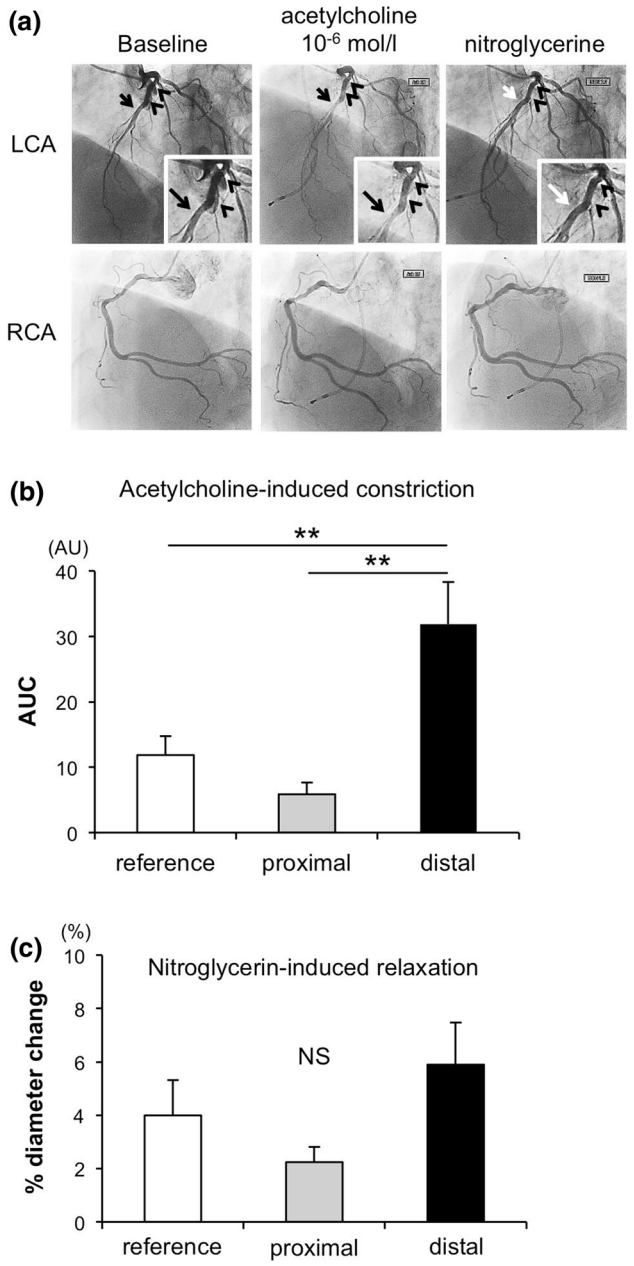

Fig. 1 **a** Representative angiograms of left coronary artery (LCA) and right coronary artery (RCA). A biolimus-eluting stent (*arrowhead*) was implanted in the proximal left anterior descending coronary artery of a 59-year-old man for effort angina. Endothelium-dependent vasoconstriction was induced by acetylcholine distal to the stent (*black arrow*) in the left anterior descending artery, but not in the RCA. Endothelium-independent vasodilatation is shown by nitroglycerine infusion (*white arrow*). **b** Changes in the area under the curve (AUC) in response to acetylcholine in the reference artery, the segment proximal to the stent, and the segment distal to the stent. $^{**}P<0.01$ versus distal to the stent. *AU* arbitrary unit. **c** Percentage of change in the vessel diameter versus baseline diameter in response to nitroglycerine infusion in the reference artery, the segment proximal to the stent, and the segment distal to the stent

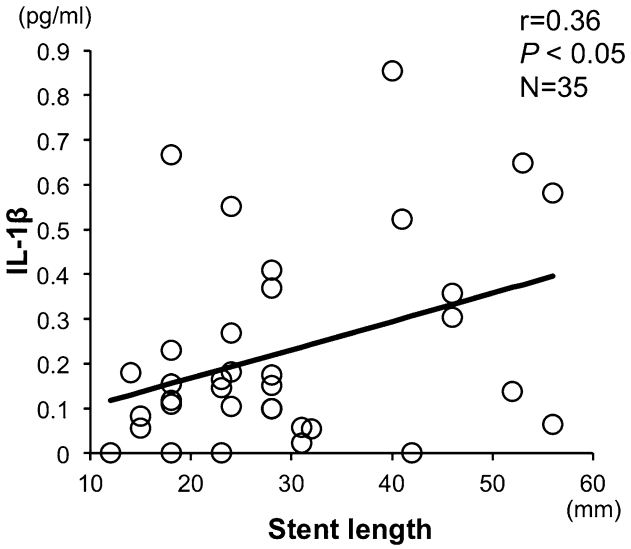

Fig. 2 Correlations between serum IL-1β levels and stent length

endothelial dysfunction at the segments distal to the stents could be detected by peripheral serum IL-1β levels.

Effects of mTOR inhibition on IL-1β release in vitro

To further determine the molecular mechanisms of endothelial dysfunction by mTOR inhibition, we examined the direct effects of sirolimus on IL-1β release, using cultured HUVECs and CASMCs. In HUVECs, IL-1β was not detected in the conditioned media at baseline and after treatment by sirolimus (10^{-6} mol/l). However, sirolimus increased IL-1β release into the conditioned media in CASMCs ($P<0.01$, sirolimus versus vehicle, Fig. 4). We then examined the effects of sirolimus on the transcriptional levels of *IL-1β* in CASMCs. Sirolimus increased *IL-1β* mRNA levels in CASMCs ($P<0.05$, sirolimus versus vehicle, Fig. 5a) and HUVECs ($P<0.05$, sirolimus versus vehicle, Fig. 5b). In contrast, sirolimus did not affect protein levels of cleaved caspase-1, an active form of caspase-1, in CASMCs (Fig. 5c). These results suggest that sirolimus increased IL-1β release via transcriptional upregulation in CASMCs.

Discussion

The novel findings of the present study are as follows. (1) Serum IL-1β levels were positively correlated with the magnitude of vasoconstriction to Ach at the segment distal to the DES. (2) Serum IL-1β levels were positively correlated with the implanted stent length. (3) An mTOR inhibitor increased mature IL-1β release in CASMCs. These findings suggest that IL-1β is released at DES stent sites and

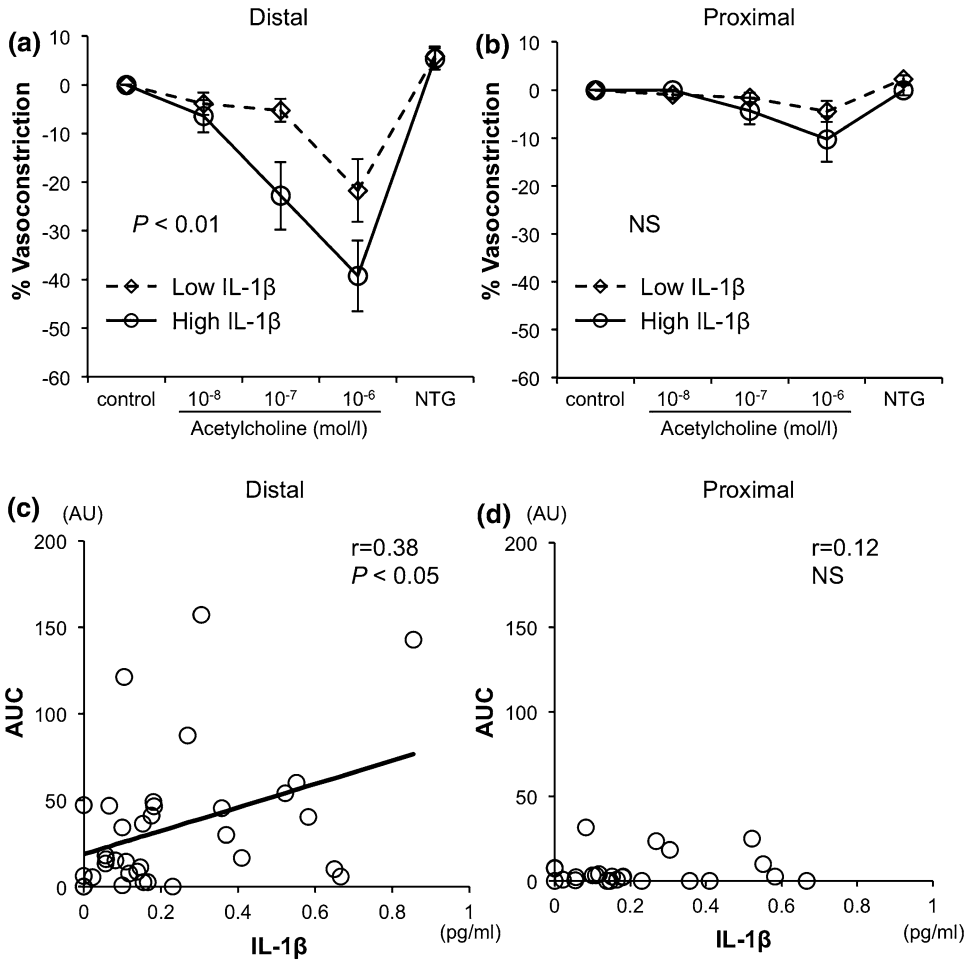

Fig. 3 Correlations between serum IL-1β levels and vasoconstriction. **a** Pooled data of acetylcholine-induced vasoconstriction at the segment distal to the stent in the low IL-1β group ($n=17$) and in the high IL-1β group ($n=18$). **b** Pooled data of acetylcholine-induced vasoconstriction at the segment proximal to the stent in the low IL-1β group ($n=12$) and in the high IL-1β group ($n=13$). **c** Correlations between serum IL-1β levels and the AUC in response to acetylcholine at the segment distal to the stent ($n=35$). **d** Correlations between serum IL-1β levels and the AUC in response to acetylcholine at the segment proximal to the stent ($n=25$). *AU* arbitrary unit

Table 3 Area under the curve and serum IL-1β levels at the segments distal to the different types of drug-eluting stents

Generation	Eluting stent	*n*	AUC (AU)	IL-1β (pg/ml)
1st	Sirolimus	2	87.5 ± 33.3	0.69 ± 0.17
2nd	Zotarolimus	1	121.2 ± 0	0.10 ± 0
2nd	Everolimus	17	18.2 ± 8.9	0.14 ± 0.04
2nd	Biolimus	15	34.0 ± 6.1	0.27 ± 0.05

Results given as mean ± SE or *n*

that circulating IL-1β levels are a biomarker of endothelial dysfunction in patients with DES implantation (Fig. 6).

IL-1β signaling might be associated with development of restenosis after stent placement [26]. However, because we excluded patients with angiographic in-stent restenosis from this study, we consider that the observed increase in IL-1β levels was not caused by restenosis. In addition, the increase of serum IL-1β levels was not associated with clinical risk factors, medications, and lesion characteristics (Table 2). In our patients, we found no correlation between serum IL-1β levels and C-reactive protein by simple linear

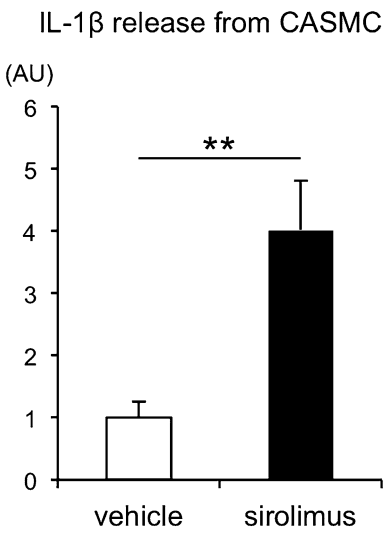

Fig. 4 mTOR inhibitor increases IL-1β release into culture media from CASMCs compared with vehicle. Pooled data show the effects of an mTOR inhibitor (sirolimus) on IL-1β concentrations in conditioned media of CASMCs. Values are mean ± SEM. $n=6$ per each group, $^{**}P<0.01$. *AU* arbitrary unit

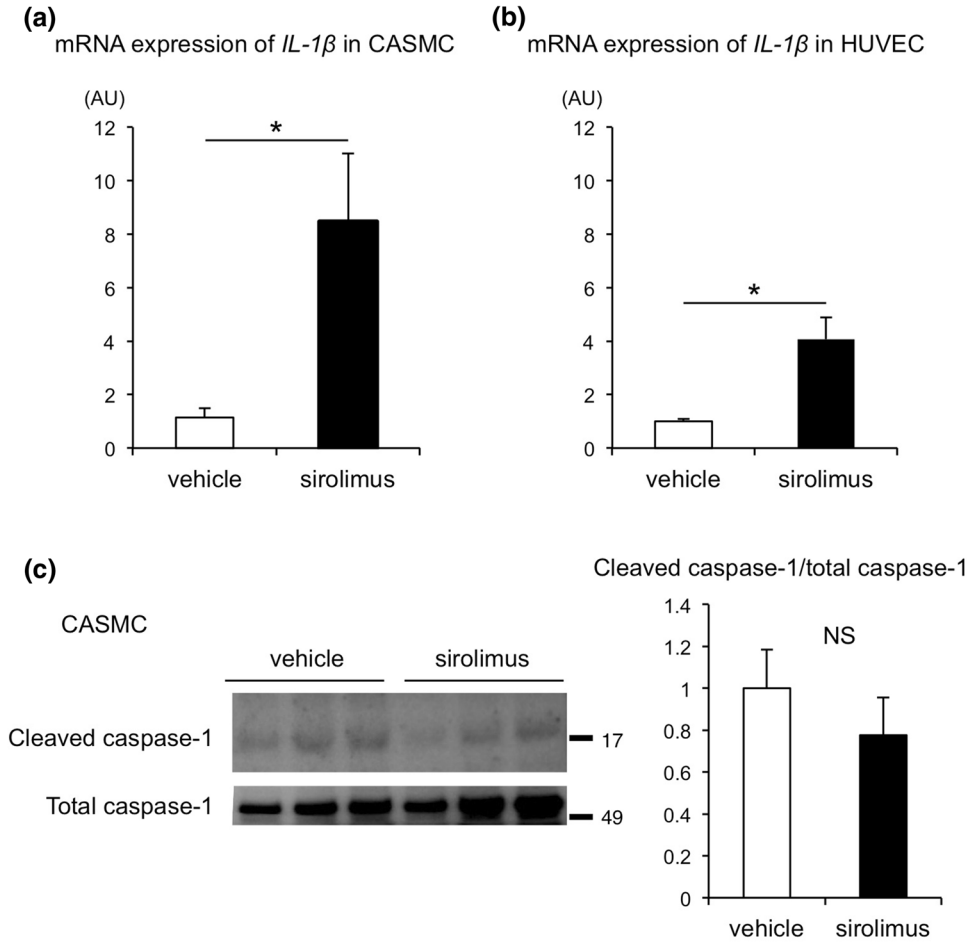

Fig. 5 mTOR inhibitor activates transcription of *IL-1β*. Pooled data show the effects of sirolimus on *IL-1β* mRNA expression levels, which were normalized to those of *GAPDH* in CASMCs (**a**) and HUVECs (**b**). $n = 7$ in each group, $^*P < 0.05$. *AU* arbitrary unit. **c** Representative immunoblots and pooled data showing the effects of sirolimus on expression levels of total caspase-1 and cleaved caspase-1 (Asp297) in CASMCs

regression analysis. This finding indicated that the increase in serum IL-1β levels in patients with risk factors was not due to inflammatory diseases.

Serum IL-1β levels and endothelial dysfunction in patients with DES implantation

Endothelial dysfunction after DES implantation has become a major concern [13, 27, 28]. Several case reports have indicated that severe coronary spasm after DES implantation is probably associated with endothelial dysfunction, leading to serious cardiac events, such as myocardial infarction [29], fatal arrhythmia, or sudden cardiac death [30]. Endothelial dysfunction after DES implantation may also contribute to late stent thrombosis, which is a life-threatening complication [10, 18, 31]. A major histological feature of late thrombosis is abnormal endothelialisation of stent struts [7, 32]. Interestingly, we observed that serum IL-1β levels were significantly correlated with the magnitude of vasoconstriction to Ach at the segments distal to DESs, but not at the proximal segments (Fig. 3a–d).

IL-1β decreases endothelial NO synthase gene expression through inhibition of p38 phosphorylation [24, 25]. IL-1β also enhances production of IL-6, C-reactive protein [33, 34], endothelin-1 [35, 36], and superoxide anion [37], which contribute to endothelial dysfunction [38]. Canakinumab is a human monoclonal antibody targeted at IL-1β. Recently, the Canakinumab Anti-inflammatory Thrombosis Outcomes Study trial has started to determine if treatment by canakinumab is effective in reducing recurrent heart attack, stroke, and cardiac death in patients with stable coronary artery disease [34].

Proinflammatory mediators contribute to endothelial dysfunction. Exposure of IL-1β to rabbit carotid arteries and a combination of tumour necrosis factor-α, interferon-γ, and lipopolysaccharide added to porcine coronary arteries markedly attenuates relaxation to Ach. This can cause abnormal vasoconstriction to serotonin or histamine [21–23]. In humans, Bhagat and colleagues demonstrated that instillation of IL-1β causes vasoconstriction in the dorsal hand vein of normal subjects [39]. Ikonomidis and colleagues showed that an IL-1 inhibitor (anakinra) improves flow-mediated, endothelium-dependent dilation of the brachial artery, coronary flow reserve, and left ventricular function in patients with rheumatoid arthritis [40]. In our study, the observed increase in serum IL-1β was not

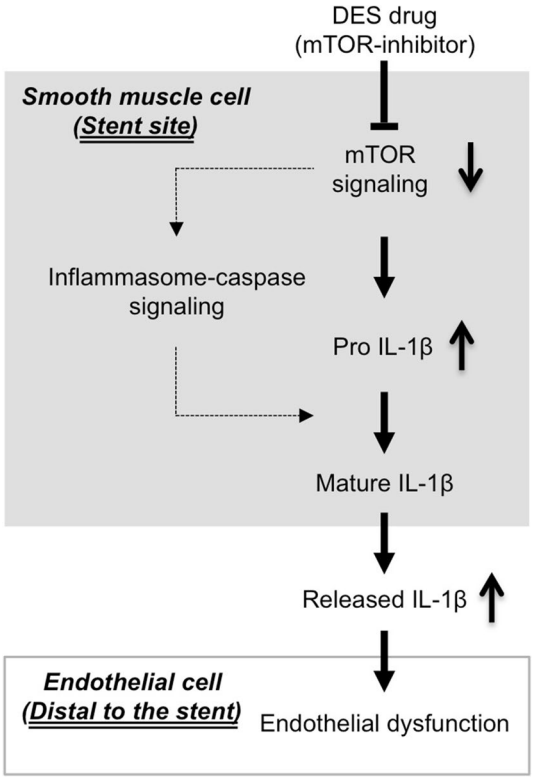

Fig. 6 Summary of the mechanisms of the mTOR-IL-1β signaling pathway in coronary endothelial dysfunction after DES implantation in the present study

caused by a systemic vascular response, because there was no relationship between serum IL-1β levels and the vasomotor response proximal to the DES site (Fig. 3b, d) and between serum IL-1β levels and vasodilation by NTG.

Carlyle et al. showed that release of sirolimus from stents could be diminished within 45–60 days after stent implantation in porcine coronary arteries [41]. However, their drug concentration was detected until 6 months in the tissue of porcine coronary arteries [41]. There are no reports of residual drug concentrations in the tissue of human coronary arteries close to the DES site. However, residual effects of the drug remaining on the distal arteries to the stent might be present for a long time.

DES length and serum IL-1β levels

The underlying mechanisms of the impaired endothelium-dependent vasomotor response distal to the DES site are not well understood. Anti-proliferative drugs are suggested to be locally diffused through the vasa vasorum to the non-stented distal segment in DES-implanted coronary arteries [42]. The length of the stented segment is independently associated with the incidence of stent thrombosis and death or myocardial infarction after DES implantation [43, 44]. The present study shows, for the first time, that serum IL-1β levels are also associated with the length of the stented segment. An increased stent site exposed by an mTOR inhibitor might release more IL-1β, which could lead to impaired endothelial function distal to the stent. In addition, increased local drug concentrations at stent regions may elicit further delay in recovery of vessels, especially impaired re-endothelialisation.

Inhibitors of mTOR and IL-1β release

Inhibitors of mTOR activate nuclear factor-kappa B and inflammasome-caspase signaling in dendritic cells [45]. However, the effect of mTOR inhibitors on endothelial cells and vascular smooth muscle cells has not been determined. To further determine the molecular mechanisms of endothelial dysfunction by mTOR inhibition, we examined the effects of sirolimus on IL-1β production using cultured HUVECs and CASMCs. We measured IL-1β release into culture media and *IL-1β* mRNA expression levels in these cells. In HUVECs, *IL-1β* mRNA expression levels were increased, although IL-1β was not detected in the conditioned media with vehicle and treatment by sirolimus. Pro-IL-1β, but not mature IL-1β, might have been formed in HUVECs. In contrast, sirolimus markedly increased mature IL-1β release into the conditioned media (Fig. 4) and *IL-1β* mRNA levels in CASMCs (Fig. 5a). The differential effects of an mTOR inhibitor on CASMCs and HUVECs should be investigated in future studies. Several studies have indicated that vascular smooth muscle cells express *IL-1β* transcripts after exposure to lipopolysaccharide [32], tumour necrosis factor-α, or IL-1 [14]. However, few studies have characterized the synthesis of mature IL-1β by vascular smooth muscle cells. The present study suggests that mTOR inhibitors trigger IL-1β release through transcriptional activation in CASMCs, which may lead to coronary endothelial dysfunction distal to the stent.

Taken together, these previous findings and our results suggest the provocative concept that serum IL-1β levels are a novel biomarker of endothelial dysfunction at the segment distal to the DES. However, the causal role of IL-1β remains unknown.

Study limitations

There are several limitations in our study. First, our study ended at 10 months after DES implantation. Therefore, whether increased serum IL-1β levels persist beyond 10 months and affect the clinical outcome remain unknown. To overcome these limitations, a prospective, randomized, multicentre, long-term, follow-up study is required. Second, we were unable to show the role of the polymer in cultured HUVECs or CASMCs. The polymer may also be

important in the process of endothelial dysfunction in DES-implanted coronary arteries. Third, we were not able to statistically analyze endothelial dysfunction and serum IL-1β levels in the different types of stent, due to the insufficient number of subjects (Table 3). Fourth, we were not able to observe a direct connection between local IL-1β levels and endothelial dysfunction in our study population. This issue also needs to be clarified in future studies. Fifth, we have no data regarding the allergic biomarkers to drug or polymer in our study population.

Conclusion

This study shows that serum IL-1β levels are associated with coronary endothelial dysfunction distal to the stent at 10 months after DES implantation.

Impact on daily practice

Our study suggests that serum IL-1β levels are a promising biomarker and a target for prevention and treatment of coronary endothelial dysfunction after DES implantation.

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Compliance with ethical standards

Conflict of interest All authors have no conflicts of interest to declare.

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References

1. Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, Colombo A, Schuler G, Barragan P, Guagliumi G, Molnar F, Falotico R, RAVEL Study Group (2002) A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. N Engl J Med 346:1773–1780
2. Moses JW, Leon MB, Popma JJ, Fitzgerald PJ, Holmes DR, O'Shaughnessy C, Caputo RP, Kereiakes DJ, Williams DO, Teirstein PS, Jaeger JL, Kuntz RE, Investigators S (2003) Sirolimus-eluting stents versus standard stents in patients with stenosis in a native coronary artery. N Engl J Med 349:1315–1323
3. Stone GW, Moses JW, Ellis SG, Schofer J, Dawkins KD, Morice MC, Colombo A, Schampaert E, Grube E, Kirtane AJ, Cutlip DE, Fahy M, Pocock SJ, Mehran R, Leon MB (2007) Safety and efficacy of sirolimus- and paclitaxel-eluting coronary stents. N Engl J Med 356:998–1008
4. Stettler C, Wandel S, Allemann S, Kastrati A, Morice MC, Schomig A, Pfisterer ME, Stone GW, Leon MB, de Lezo JS, Goy JJ, Park SJ, Sabate M, Suttorp MJ, Kelbaek H, Spaulding C, Menichelli M, Vermeersch P, Dirksen MT, Cervinka P, Petronio AS, Nordmann AJ, Diem P, Meier B, Zwahlen M, Reichenbach S, Trelle S, Windecker S, Juni P (2007) Outcomes associated with drug-eluting and bare-metal stents: a collaborative network meta-analysis. Lancet 370:937–948
5. Bonaa KH, Mannsverk J, Wiseth R, Aaberge L, Myreng Y, Nygard O, Nilsen DW, Klow NE, Uchto M, Trovik T, Bendz B, Stavnes S, Bjornerheim R, Larsen AI, Slette M, Steigen T, Jakobsen OJ, Bleie O, Fossum E, Hanssen TA, Dahl-Eriksen O, Njolstad I, Rasmussen K, Wilsgaard T, Nordrehaug JE, Investigators N (2016) Drug-eluting or bare-metal stents for coronary artery disease. N Engl J Med 375:1242–1252
6. Yano H, Horinaka S, Ishikawa M, Ishimitsu T (2016) The efficacy of everolimus-eluting stent implantation in patients with ST-segment elevation myocardial infarction: outcomes of 2-year clinical follow-up. Heart Vessels 31:1609–1615
7. Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, Kutys R, Skorija K, Gold HK, Virmani R (2006) Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. J Am Coll Cardiol 48:193–202
8. Luscher TF, Steffel J, Eberli FR, Joner M, Nakazawa G, Tanner FC, Virmani R (2007) Drug-eluting stent and coronary thrombosis: biological mechanisms and clinical implications. Circulation 115:1051–1058
9. Nasuno T, Tokura M, Kageyama M, Toyoda S, Sakuma M, Komatsu T, Taguchi I, Abe S, Inoue T (2016) The wound healing response after implantation of a drug-eluting stent is impaired persistently in the long term. Heart Vessels 31:985–989
10. Mitsutake Y, Ueno T, Yokoyama S, Sasaki K, Sugi Y, Toyama Y, Koiwaya H, Ohtsuka M, Nakayoshi T, Itaya N, Chibana H, Kakuma T, Imaizumi T (2012) Coronary endothelial dysfunction distal to stent of first-generation drug-eluting stents. JACC Cardiovasc Interv 5:966–973
11. Terashima M, Kaneda H, Nasu K, Matsuo H, Habara M, Ito T, Tanaka N, Rathore S, Kinoshita Y, Kimura M, Ehara M, Suzuki Y, Suzuki T (2012) Protective effect of telmisartan against endothelial dysfunction after coronary drug-eluting stent implantation in hypertensive patients. JACC Cardiovasc Interv 5:182–190
12. Mischie AN, Nazzaro MS, Fiorilli R, De Felice F, Musto C, Confessore P, Parma A, Boschetti C, Violini R (2013) Head-to-head comparison of sirolimus-eluting stent versus bare metal stent evaluation of the coronary endothelial dysfunction in the same patient presenting with multiple coronary artery lesions: the CREDENTIAL study. Catheter Cardiovasc Interv 82:E184–E191
13. Hofma SH, van der Giessen WJ, van Dalen BM, Lemos PA, McFadden EP, Sianos G, Ligthart JM, van Essen D, de Feyter PJ, Serruys PW (2006) Indication of long-term endothelial dysfunction after sirolimus-eluting stent implantation. Eur Heart J 27:166–170
14. Aizawa K, Yasuda S, Takahashi J, Takii T, Kikuchi Y, Tsuburaya R, Ito Y, Ito K, Nakayama M, Takeda M, Shimokawa H (2012) Involvement of Rho-kinase activation in the pathogenesis of coronary hyperconstricting responses induced by drug-eluting stents in patients with coronary artery disease. Circ J 76:2552–2560
15. Mitsutake Y, Ueno T, Ikeno F, Yokoyama S, Sasaki K, Nakayoshi T, Itaya N, Chibana H, Sasaki M, and Fukumoto Y (2016) Serial changes of coronary endothelial function and arterial healing after paclitaxel-eluting stent implantation. Cardiovasc Interv Ther 31:21–28
16. Mitsutake Y, Ueno T, Ikeno F, Yokoyama S, Sasaki K, Ohtsuka M, Nakayoshi T, Itaya N, Chibana H, Sasaki M, Fukumoto Y (2015) Second-generation everolimus-eluting stents demonstrate better vascular function, less thrombus formation, and less

yellow intima than first-generation drug-eluting stents. AsiaIntervention 1:33–40
17. Hamilos MI, Ostojic M, Beleslin B, Sagic D, Mangovski L, Stojkovic S, Nedeljkovic M, Orlic D, Milosavljevic B, Topic D, Karanovic N, Wijns W, and Investigators NC (2008) Differential effects of drug-eluting stents on local endothelium-dependent coronary vasomotion. J Am Coll Cardiol 51:2123–2129
18. Togni M, Windecker S, Cocchia R, Wenaweser P, Cook S, Billinger M, Meier B, Hess OM (2005) Sirolimus-eluting stents associated with paradoxic coronary vasoconstriction. J Am Coll Cardiol 46:231–236
19. Martinon F, Burns K, Tschopp J (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 10:417–426
20. Cogswell JP, Godlevski MM, Wisely GB, Clay WC, Leesnitzer LM, Ways JP, Gray JG (1994) NF-kappa B regulates IL-1 beta transcription through a consensus NF-kappa B binding site and a nonconsensus CRE-like site. J Immunol 153:712–723
21. Kessler P, Bauersachs J, Busse R, Schini-Kerth VB (1997) Inhibition of inducible nitric oxide synthase restores endothelium-dependent relaxations in proinflammatory mediator-induced blood vessels. Arterioscler Thromb Vasc Biol 17:1746–1755
22. Fukumoto Y, Shimokawa H, Kozai T, Kadokami T, Kuwata K, Yonemitsu Y, Kuga T, Egashira K, Sueishi K, Takeshita A (1997) Vasculoprotective role of inducible nitric oxide synthase at inflammatory coronary lesions induced by chronic treatment with interleukin-1beta in pigs in vivo. Circulation 96:3104–3111
23. Fukumoto Y, Shimokawa H, Ito A, Kadokami T, Yonemitsu Y, Aikawa M, Owada MK, Egashira K, Sueishi K, Nagai R, Yazaki Y, Takeshita A (1997) Inflammatory cytokines cause coronary arteriosclerosis-like changes and alterations in the smooth-muscle phenotypes in pigs. J Cardiovasc Pharmacol 29:222–231
24. Niwano K, Arai M, Koitabashi N, Hara S, Watanabe A, Sekiguchi K, Tanaka T, Iso T, Kurabayashi M (2006) Competitive binding of CREB and ATF2 to cAMP/ATF responsive element regulates eNOS gene expression in endothelial cells. Arterioscler Thromb Vasc Biol 26:1036–1042
25. Yamagata K, Tanaka N, Matsufuji H, Chino M (2012) Beta-carotene reverses the IL-1beta-mediated reduction in paraoxonase-1 expression via induction of the CaMKKII pathway in human endothelial cells. Microvasc Res 84:297–305
26. Kastrati A, Koch W, Berger PB, Mehilli J, Stephenson K, Neumann FJ, von Beckerath N, Bottiger C, Duff GW, Schomig A (2000) Protective role against restenosis from an interleukin-1 receptor antagonist gene polymorphism in patients treated with coronary stenting. J Am Coll Cardiol 36:2168–2173
27. Shin DI, Seung KB, Kim PJ, Chang K, Choi JK, Jeon DS, Kim MJ, Lee MY, Chung WS (2008) Long-term coronary endothelial function after zotarolimus-eluting stent implantation. A 9 month comparison between zotarolimus-eluting and sirolimus-eluting stents. Int Heart J 49:639–652
28. Kim JW, Seo HS, Park JH, Na JO, Choi CU, Lim HE, Kim EJ, Rha SW, Park CG, Oh DJ (2009) A prospective, randomized, 6-month comparison of the coronary vasomotor response associated with a zotarolimus- versus a sirolimus-eluting stent: differential recovery of coronary endothelial dysfunction. J Am Coll Cardiol 53:1653–1659
29. Kim JW, Park CG, Seo HS, Oh DJ (2005) Delayed severe multivessel spasm and aborted sudden death after Taxus stent implantation. Heart 91:e15
30. Brott BC, Anayiotos AS, Chapman GD, Anderson PG, Hillegass WB (2006) Severe, diffuse coronary artery spasm after drug-eluting stent placement. J Invasive Cardiol 18:584–592
31. Pendyala LK, Yin X, Li J, Chen JP, Chronos N, Hou D (2009) The first-generation drug-eluting stents and coronary endothelial dysfunction. JACC Cardiovasc Interv 2:1169–1177
32. Shiroto T, Yasuda S, Tsuburaya R, Ito Y, Takahashi J, Ito K, Ishibashi-Ueda H, Shimokawa H (2009) Role of Rho-kinase in the pathogenesis of coronary hyperconstricting responses induced by drug-eluting stents in pigs in vivo. J Am Coll Cardiol 54:2321–2329
33. Sironi M, Breviario F, Proserpio P, Biondi A, Vecchi A, Van Damme J, Dejana E, Mantovani A (1989) IL-1 stimulates IL-6 production in endothelial cells. J Immunol 142:549–553
34. Ridker PM, Thuren T, Zalewski A, Libby P (2011) Interleukin-1beta inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). Am Heart J 162:597–605
35. Corder R, Carrier M, Khan N, Klemm P, Vane JR (1995) Cytokine regulation of endothelin-1 release from bovine aortic endothelial cells. J Cardiovasc Pharmacol 26(Suppl 3):S56–S58
36. Klemm P, Warner TD, Corder R, Vane JR (1995) Endothelin-1 mediates coronary vasoconstriction caused by exogenous and endogenous cytokines. J Cardiovasc Pharmacol 26(Suppl 3):S419–S421
37. Takahashi T, Nishizawa Y, Hato F, Shintaku H, Maeda N, Fujiwara N, Inaba M, Kobayashi K, Kitagawa S (2007) Neutrophil-activating activity and platelet-activating factor synthesis in cytokine-stimulated endothelial cells: reduced activity in growth-arrested cells. Microvasc Res 73:29–34
38. Haruna Y, Morita Y, Komai N, Yada T, Sakuta T, Tomita N, Fox DA, Kashihara N (2006) Endothelial dysfunction in rat adjuvant-induced arthritis: vascular superoxide production by NAD(P)H oxidase and uncoupled endothelial nitric oxide synthase. Arthritis Rheum 54:1847–1855
39. Bhagat K, Vallance P (1997) Inflammatory cytokines impair endothelium-dependent dilatation in human veins in vivo. Circulation 96:3042–3047
40. Ikonomidis I, Lekakis JP, Nikolaou M, Paraskevaidis I, Andreadou I, Kaplanoglou T, Katsimbri P, Skarantavos G, Soucacos PN, Kremastinos DT (2008) Inhibition of interleukin-1 by anakinra improves vascular and left ventricular function in patients with rheumatoid arthritis. Circulation 117:2662–2669
41. Carlyle WC, McClain JB, Tzafriri AR, Bailey L, Zani BG, Markham PM, Stanley JR, Edelman ER (2012) Enhanced drug delivery capabilities from stents coated with absorbable polymer and crystalline drug. J Control Release 162:561–567
42. Sahler LG, Davis D, Saad WE, Patel NC, Lee DE, Waldman DL (2008) Comparison of vasa vasorum after intravascular stent placement with sirolimis drug-eluting and bare metal stents. J Med Imaging Radiat Oncol 52:570–575
43. Suh J, Park DW, Lee JY, Jung IH, Lee SW, Kim YH, Lee CW, Cheong SS, Kim JJ, Park SW, Park SJ (2010) The relationship and threshold of stent length with regard to risk of stent thrombosis after drug-eluting stent implantation. JACC Cardiovasc Interv 3:383–389
44. Caputo RP, Goel A, Pencina M, Cohen DJ, Kleiman NS, Yen CH, Waksman R, Tolerico P, Dhar G, Gordon P, Bach RG, Lopez JJ (2012) Impact of drug eluting stent length on outcomes of percutaneous coronary intervention (from the EVENT registry). Am J Cardiol 110:350–355
45. Ohtani M, Nagai S, Kondo S, Mizuno S, Nakamura K, Tanabe M, Takeuchi T, Matsuda S, Koyasu S (2008) Mammalian target of rapamycin and glycogen synthase kinase 3 differentially regulate lipopolysaccharide-induced interleukin-12 production in dendritic cells. Blood 112:635–643