European Heart Journal Advance Access published February 27, 2008

European Heart Journal
doi:10.1093/eurheartj/ehn060

CLINICAL RESEARCH

Vascular endothelial growth factor protein levels and gene expression in peripheral monocytes after stenting: a randomized comparative study of sirolimus: eluting and bare metal stents

George E. Kochiadakis[1], Maria E. Marketou[1], Dimitris Panutsopulos[2], Dimitris A Arfanakis[1], Emmanuel I. Skalidis[1], Nikolaos E. Igoumenidis[1], Michael I. Hamilos[1], George Sourvinos[2], Gregory Chlouverakis[3], Demetrios Spandidos[2], and Panos E. Vardas[1]*

[1]Cardiology Department, Heraklion University Hospital, PO Box 1352, Heraklion 71110, Crete, Greece; [2]Laboratory of Virology, University of Crete, Crete, Greece; and [3]Division of Biostatistics, University of Crete, Crete, Greece

Received 6 October 2007; revised 16 January 2008; accepted 24 January 2008

Aims Although previous studies have indicated that vascular endothelial growth factor (VEGF) plays an important role in the vascular-healing process after stent implantation, its effect on in-stent restenosis is unclear. We assessed VEGF serum protein levels and gene expression in peripheral monocytes in relation to in-stent restenosis after implantation of sirolimus-eluting (SES) and bare metal stents (BMS) in a non-blinded, randomized study.

Methods and results Forty-two patients (28 men, age 62 $\pm$ 11 years) with stable angina, who underwent elective single-vessel percutaneous coronary intervention, were randomized to SES ($n = 21$) or BMS ($n = 21$) implantation. VEGF protein levels in the BMS group showed an increasing trend ($P = 0.083$), whereas in the SES group they decreased significantly ($P = 0.002$). BMS induced up-regulation of VEGF mRNA levels, whereas for SES down-regulation was observed. There was no correlation between serum levels and late luminal loss. A significant correlation was found between VEGF gene expression and late luminal loss in both groups (BMS: $r = 0.98$, $P < 0.001$; SES: $r = 0.65$, $P = 0.002$).

Conclusion SES, in comparison with BMS, results in lower VEGF protein levels and gene expression in peripheral monocytes. The latter shows a positive relationship with in-stent late-luminal loss, suggesting an essential role in the reduced in-stent restenosis seen in SES.

Keywords Vascular endothelial growth factor • Sirolimus-eluting stents • Restenosis

Introduction

Despite the widening use of drug-eluting stents (DES), in-stent restenosis continues to pose a problem. These stents may substantially reduce the incidence of in-stent restenosis compared with bare metal stents (BMS), but they do not eliminate it altogether.[1] Placing a foreign body, such as a metallic stent, within the coronary vessels causes injury and leads to various biological changes in the vascular wall, as well as in circulating blood cells such as monocytes and platelets.[2,3] Through the action of complex mechanisms that are still not fully understood, this results in the creation of intimal hyperplasia and in-stent restenosis.

Previous studies have reported that vascular endothelial growth factor (VEGF)—a molecule with strong angiogenic properties—plays an important role in the vascular-healing process after stent implantation,[4] although it has not been determined whether this role is beneficial or not. Furthermore, studies to date have been of BMS, so we do not know what role VEGF

*Corresponding author. Tel: +30 2810 392877. Email: cardio@med.uoc.gr

plays in the healing process after DES deployment and whether it is related with the reduced restenosis rates these newer stents exhibit.

Sirolimus-eluting stents (SES) are among the most effective DES.[5] In this study we investigated the changes of VEGF serum levels and VEGF gene expression in peripheral monocytes after stenting in patients with stable angina and evaluated their relationship with the in-stent late luminal loss over a 6 month follow-up after BMS or SES implantation. We focused on peripheral blood monocytes since these cells are closely involved with in-stent restenosis and are one of the most important sources of VEGF.[2,6]

Methods

Study population

Forty-two patients (28 men) aged <70 years old (mean 62 ± 11 years) were enrolled from 720 consecutive patients who underwent successful elective coronary angioplasty with coronary stent implantation in the Cardiology department of Heraklion University Hospital from April 2005 to January 2006. Inclusion criteria were stable angina lasting >6 months and severe stenosis of one major epicardial coronary artery (>70% narrowing of the lumen with vessel reference diameter ≥2.5 mm).

All patients had viable myocardium at risk, defined by the development of typical chest pain with ST-segment depression ≥1 mm during an exercise stress test, or a reversible defect on exercise thallium^{201} myocardial perfusion scintigraphic study.

Patients with evidence of a previous myocardial infarction, cardiogenic shock, prior percutaneous coronary intervention or bypass surgery, as well as patients with in-stent restenosis, disease of the left main coronary artery, chronic total occlusion (>3 months), bifurcation stenting, or adjacent stented segments >25 mm were excluded. Other exclusion criteria were treatment with anti-inflammatory or immunosuppressive drugs except for aspirin, allergy to aspirin, heparin, or clopidogrel, significant valvular disease, myocarditis, and history or signs of neoplastic or haematological disease, heart, renal or hepatic failure, and history of any inflammatory disease during the last 6 months. Any cardiovascular medication had been unchanged for 3 months prior to the study in all patients and was maintained throughout the study period.

Patients were non-blindly randomly allocated by a computer algorithm to one of two groups, for SES ($n = 21$) or BMS ($n = 21$) implantation (*Figure 1*). The randomization used an SPSS routine that selected 21 out of 42 subjects who would receive SES. The remaining 21 comprised the BMS group. The allocation sequence was not accessible to the doctors performing the stent implantation. After an eligible patient gave informed consent, the doctor performing the procedure telephoned the statistician, who kept the randomized list in a locked drawer and revealed the type of stent that should be used.

All patients were started on treatment with aspirin 325 mg and clopidogrel 300 mg before the deployment of an intracoronary stent, followed by 75 mg clopidogrel and 100 mg aspirin daily during the follow-up. Angioplasty and stent implantation were performed after an intravenous bolus of 80 IU/kg of heparin, according to the standard technique.

Patients visited the outpatient clinic of the cardiology department at the end of the first month after the procedure and then every 2 months, or when angina-like symptoms occurred. On each visit the patients had a clinical examination and underwent routine laboratory exams and an exercise test when this was considered advisable. All patients were asked to return for coronary angiography between 6 and 8 months after randomization, or earlier if they had findings suggestive of myocardial ischaemia, including angina, or not.

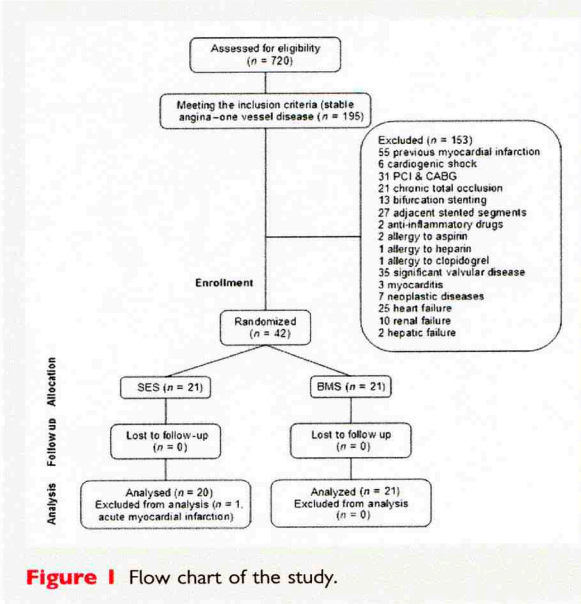

Figure 1 Flow chart of the study.

The study was approved by the hospital's ethics committee; all institutional guidelines were followed and all participants gave written informed consent.

Study endpoints

The primary endpoint was the post-interventional comparison of VEGF serum levels between BMS and SES. More specifically, we hypothesized that a difference of, at least, 45 pg/mL between the two groups was worth detecting. In parallel we evaluated the changes of VEGF gene expression in peripheral monocytes after BMS or SES implantation.

Secondary endpoints included in-stent late luminal loss at 6 months and its relationship with the changes of VEGF serum levels and VEGF gene expression after stent implantation.

In addition, to evaluate the role of rapamycin (the drug that coated the SES) in VEGF changes in a human monocytic-like myeloma cell line, THP-1, we assessed in an *in vitro* study the effects of different rapamycin levels on VEGF gene expression and VEGF protein secretion.

Blood collection and monocyte separation

Blood samples were obtained 24 h before (baseline blood samples taken from the resting patient after a 14 h overnight fast) and on the first visit 1 month after the coronary intervention. The blood was processed according to a previously described procedure[7] and the mononuclear cells were isolated. $CD14^+$ monocytes were purified from mononuclear cells by positive selection using MACS high gradient magnetic separation columns type MS and negative magnetic bead selection. Purity assessed by FACS (FACS Calibur, Beckton Dickinson) analysis was >95%.

Experimental study

THP-1 culture

THP-1 cells were cultured as previously described.[8] The cells were divided into two groups, in cell culture wells, starting from a single

initial population and cultured in parallel, under the same conditions. One group was activated by *Salmonella enteritidis*-derived lipopolysaccharide (LPS), serotype 0111:B4 (SIGMA) but the other was not. Each group was further divided into subgroups: non-treated and treated with rapamycin (SIGMA) at the corresponding concentrations (0.1, 1, 10, and 100 ng/mL). In each subgroup the cells were cultured for different time periods (3, 12, and 24 h) as indicated in each experiment. For each assay, e.g. activation by LPS, rapamycin, etc., also including the appropriate matched controls, 4×10^5 cells were seeded onto six-well plates and treated accordingly. Three independent experimental studies were performed.

MTT assay

The effects of rapamycin on cell viability were estimated by MTT ([3-(4,5-dimethylthiazol-2yl)-2,6-dimethyl-morpholino)-2,5-diphenyl-tetrazolium bromide]) viability as described previously.[9] The cells were divided into groups according to the study design and were cultured for different time periods. Viability for the LPS-activated and non-activated cells at different rapamycin levels was defined as the treated to the corresponding untreated absorbance ratio. Again three independent experimental studies were performed.

Extraction and quantification of mRNA and real-time semi-quantitative polymerase chain reaction

Total RNA was isolated directly from the isolated monocytes and THP-1 cells using the Trizol reagent (Life Technologies Ltd., UK) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized with a ThermoScript™ RT system (Invitrogen Corp, CA) using 1.5 μg of total RNA according to the manufacturer's protocol. The cDNA was used as a template for target amplification using Assays-on-Demand Gene Expression Products (Applied Biosystems). Expression levels of VEGF were normalized using GAPDH. The primer sets for VEGF (Hs00173626_m1) and GAPDH (Hs999999.05_m1) were purchased from Applied Biosystems. mRNA levels for specific gene products were subsequently determined by real-time semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis with an ABI Prism 7000 Sequence Detection System (Applied Biosystems). Reactions were as follows: 50°C for 2 min, 95°C for 10 min, and then 40 cycles of 95°C for 15 s and 60°C for 1 min. Dual negative controls consisting of no template and extraction buffer as template were included with each experiment.

mRNA levels were estimated by comparing the threshold cycle value with that of the cDNA standard curve. The fold inductions of mRNA were then calculated as follows: (a) for the *in vivo* study, fold induction was the ratio of mRNA levels after stent implantation to those before. (b) For the *in vitro* study, fold induction was the ratio of mRNA at different rapamycin levels of the activated and non-activated cells to the corresponding non-treated values. Thus mRNA folds equal to one indicates no change. VEGF protein levels in supernatants and serum were measured with a commercially available ELISA kit (Quantikine R&D Systems).

Statistical analysis

As mentioned above, the primary endpoint was the post-interventional comparison of VEGF serum levels in the BSM and SES groups. Sample size calculation showed that to achieve 80% power to detect as significant at the 5% level, a difference of at least 45 pg/mL in VEGF protein levels between the two groups, a total of 42 patients, was needed. The assumed common standard deviation was 50 pg/mL.

Summary descriptive statistics are reported as mean ± SD for continuous variables or percentages for categorical ones. Clinical and demographic variables were compared between the BMS and SES groups, with two-sided *t*-tests or non-parametric Mann–Whitney tests, Fisher's exact test, as appropriate. Kolmogorov–Smirnov tests were used to examine whether the distributions of VEGF levels and late luminal loss showed significant deviations from normality. Since VEGF levels were measured at two time points in the same patient, repeated measures analysis of variance (ANOVA) was used to assess changes in VEGF levels from pre to post between the two groups. Repeated measures ANOVA was also used to determine whether smoking, diabetes mellitus, hyperlipidemia, hypertension, or angiographic or procedural characteristics, such as the vessel size, type of lesion, length of stented segment, or in-stent late luminal loss influenced the time course of VEGF protein levels. Where findings for any variable were significant *post hoc* Bonferroni adjusted tests were used to pinpoint differences. mRNA fold induction was compared between groups with Mann–Whitney test. 95% confidence intervals (CI) for mRNA fold induction and viability of cells were computed to provide additional information about the magnitude of true mean differences. Associations between VEGF levels and late luminal loss were assessed with standard linear regression and correlation methods. A two-sided *P* value less than 5% was the criterion for significance. SPSS 13 was the statistical package used for all analyses.

Results

The procedure was successful in all patients, and no significant complications occurred. Demographic and clinical data, angiographic findings, and procedural variables were similar among the two groups (*Table 1*). There were no significant changes in the patients' laboratory tests (haematocrit, platelet count, cholesterol levels, etc.) during the study (see Supplementary material online).

The median follow-up was 6 months (interquartile range 1 month). One patient in the SES group had an acute anterior myocardial infarction on the 10th day after stent implantation as a result of acute in-stent thrombosis and was excluded from our study. No other patient complained of any clinical symptom before the end of the first month. Five patients (four in the BMS group and one in the SES group) exhibited symptoms suggestive of angina and/or signs of ischaemia during a later exercise test and had a coronary angiographic examination before the end of the 6 months. All of the four in the BMS group showed in-stent restenosis (≥50% stenosis of total lumen diameter at follow-up), whereas the patient in the SES group did not. The latter patient was treated with calcium channel blockers, which led to relief of symptoms, and the thallium scintigraphy at six months follow-up was negative. All the remaining patients had follow-up angiography between 6 and 8 months after randomization and none showed restenosis. The minimum luminal diameter at follow-up was significantly greater and the in-stent late luminal loss smaller in the SES compared with the BMS group (2.24 ± 0.32 vs. 1.81 ± 0.48 mm, $P = 0.005$, and 0.58 ± 0.25 vs. 1.11 ± 0.61 mm, $P = 0.001$, respectively).

Serum VEGF protein levels after stent implantation

Repeated measures ANOVA showed a significant stent–VEGF protein serum level interaction ($P = 0.001$). More precisely, initial

Table 1 Demographic and clinical data, angiographic findings, and procedural variables in BMS and SES group

	BMS ($n = 21$)	SES ($n = 20$)
Age (years)	63 (10)	60 (11)
Male	13 (65%)	14 (70%)
Smoking	11 (52%)	10 (50%)
Diabetes mellitus	12 (57%)	8 (40%)
Hypertension	15 (71%)	10 (50%)
Hyperlipidemia	18 (86%)	16 (80%)
Beta-blockers	10 (48%)	11 (55%)
ACE-i	8 (38%)	6 (30%)
Statins	19 (90%)	17 (85%)
Left anterior descending artery	10 (48%)	9 (45%0
Right coronary artery	6 (29%)	5 (25%)
Circumflex	5 (24%)	6 (30%)
ACC/AHA type of lesion		
Lesion type A	9 (43%)	7 (35%)
Lesion type B	10 (48%)	10 (50%)
Lesion type C	2 (10%)	3 (15%)
Vessel size (mm)	2.94 (0.4)	2.89 (0.56)
Minimum luminal diameter before stenting (mm)	0.75 (0.38)	0.80 (0.37)
Acute gain	2.13 (0.4)	2.07 (0.39)
Stent length (mm)	24 (11)	21 (10)
Minimum luminal diameter after stenting (mm)	2.93 (0.36)	2.82 (0.41)

BMS, bare metal stents; SES, sirolimus-eluting stents; ACE-i, angiotensin-converting enzyme inhibitors.

mean VEGF protein levels were not significantly different in the two groups (386 ± 44 pg/mL in BMS group vs. 403 ± 45 pg/mL in SES group, $P = 0.10$), but the BMS group showed an increasing trend (to 458 ± 38 pg/mL, $P = 0.083$), whereas in the SES group the levels decreased significantly (to 296 ± 40 pg/mL, $P = 0.002$). Consequently, serum protein levels of VEGF differed significantly between the two groups 1 month after stent implantation ($P = 0.006$).

None of the patients' characteristics studied, such as smoking, diabetes mellitus, hyperlipidemia, hypertension, or angiographic or procedural characteristics, such as the vessel size, type of lesion, length of stented segment, or in-stent late luminal loss, was associated with the change of VEGF serum protein levels in either group.

VEGF gene expression in peripheral monocytes after stent implantation

Baseline VEGF mRNA levels in peripheral monocytes did not differ between the two groups (9.8 ± 6.69 in BMS vs. 13.3 ± 6.7 in SES, $P = 0.79$). BMS implantation induced an up regulation of VEGF mRNA levels, in contrast to SES where a down-regulation was observed (fold induction: 1.44 ± 0.84 in BMS group vs. 0.72 ± 0.35 in SES group, $P = 0.001$). None of the patients' characteristics,

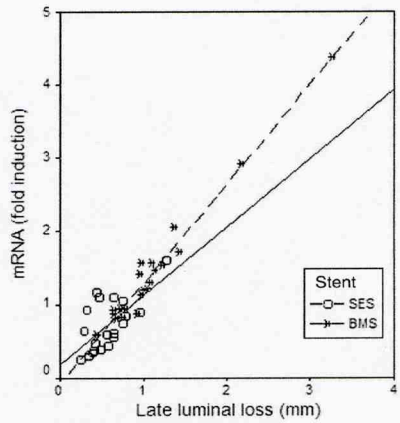

Figure 2 Scatter plot of changes in VEGF gene expression (fold induction) in peripheral monocytes and in-stent late lumen loss at a 6 month follow-up. A strong linear relationship is apparent in both groups. VEGF, vascular endothelial growth factor.

angiographic, or procedural data was associated with changes in VEGF gene expression as measured by fold induction in either group. However, we found a significant correlation between fold induction and in-stent late luminal loss at 6 month follow-up in both groups (BMS: $r = 0.98$, $P < 0.001$, SES: $r = 0.65$, $P = 0.002$, *Figure 2*).

Effect of rapamycin on THP-1 monocytes

To determine whether rapamycin is important in the down-regulation of VEGF expression in peripheral monocytes, an *in vitro* THP-1 monocytes cell culture was performed. In these experiments the effect of different rapamycin levels on mRNA folds at various time points, in THP-1 monocytes with and without LPS stimulation, was as shown in *Figure 3*.

In both groups a significant decrease was observed in the gene expression of VEGF, which was already apparent during the first 3 h of cell culture. In cells with LPS activation at this time point the significant decrease from baseline was seen at a rapamycin concentration of 1 ng/mL [95% CI of fold induction: (0.16–0.96)] while in cells without LPS activation it was seen at 100 ng/mL [95% CI of fold induction: (−0.03 to 0.79)].

At 12 h the significant drop in VEGF gene expression in non-activated cells was also achieved with 1 ng/mL [95% CI of fold induction (0.12–0.90)], whereas in LPS-activated cells at 24 h it was achieved with 0.1 ng/mL [95% CI of fold induction: (0.63–0.89)].

A decrease in the protein levels from baseline was also observed at the same concentration levels, but became significant at later time points (12 h) in both groups (mean difference in LPS activated cells at 1 ng/mL: 88.7 ± 10 pg/mL, $P = 0.005$, and in the group without LPS activation at 100 ng/mL: 71.5 ± 14 pg/mL, $P = 0.012$) (*Figure 4*).

To further investigate whether the various rapamycin concentrations in the THP-1 cell-culture influenced the viability of the cells and consequently the VEGF mRNA and protein levels, an

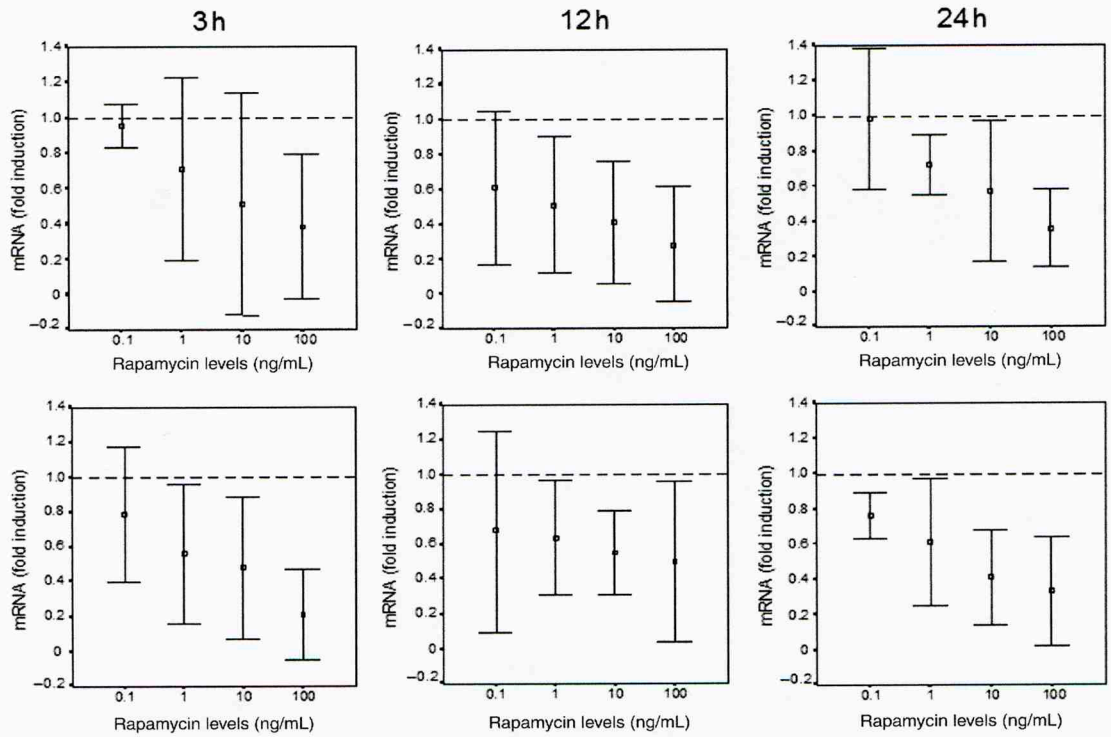

Figure 3 95% CI for mRNA (fold induction) in LPS activated and non-activated cells at different rapamycin concentrations and various time points. Error bars that fall below one indicate significant change with respect to untreated cells. Upper row, LPS (−); lower row, LPS (+); LPS, lipopolysachharide.

MTT-assay was performed. The viability of cells at the same rapamycin concentrations and time points is shown in *Figure 5*. A significant decline was observed only at 100 ng/mL rapamycin and was already apparent from the first 3 h in both groups [95% CI of viability for LPS activated cells: (0.27–0.95) and for cells without LPS activation: (0.24–0.98)].

Discussion

VEGF is an important angiogenic cytokine that has been directly linked to both physiological and pathophysiological angiogenesis.[10] Previous experimental studies reported that VEGF mRNA and VEGF protein were present after stent deployment and were prominent in proliferated smooth muscle cells, in the infiltrating macrophages, and in the endothelial cells of microvessels around the stent struts.[11]

However, the role of VEGF in in-stent restenosis has not been completely clarified. For this reason we designed this study, in which we assessed and compared the behaviour of VEGF circulating protein levels and VEGF gene expression in peripheral monocytes after both BMS and SES implantation in living humans and evaluated their relation with neointimal growth.

SES have come onto the scene in recent years.[5] These stents have been shown to limit restenosis and demonstrate a significant reduction in vasculoproliferative response following post-angioplasty endothelial injury.[12] Although sirolimus (rapamycin) is a known inhibitor of regulatory elements of the cell cycle, its precise effect on vascular-healing properties and thus restenosis needs further investigation.

Effects of BMS on VEGF serum protein levels and VEGF gene expression in monocytes

Our study showed first of all that after BMS implantation VEGF serum protein levels show a tendency to rise, and that this increase does not appear to be significantly correlated with the in-stent late luminal loss at 63 month follow-up. These results suggest that in humans, and under *in vivo* conditions, VEGF may play a part in the process of re-endothelization and repair of the arterial wall after BMS deployment, as previous studies in animals have shown; however, it does not appear to promote restenosis after stenting. Furthermore, our study showed that the VEGF gene expression in peripheral monocytes increases after BMS deployment, and that this increase has a strong positive correlation with the in-stent late luminal loss. This finding complements and reinforces the results of earlier studies. Indeed, previous studies have reported that circulating monocytes increased after coronary

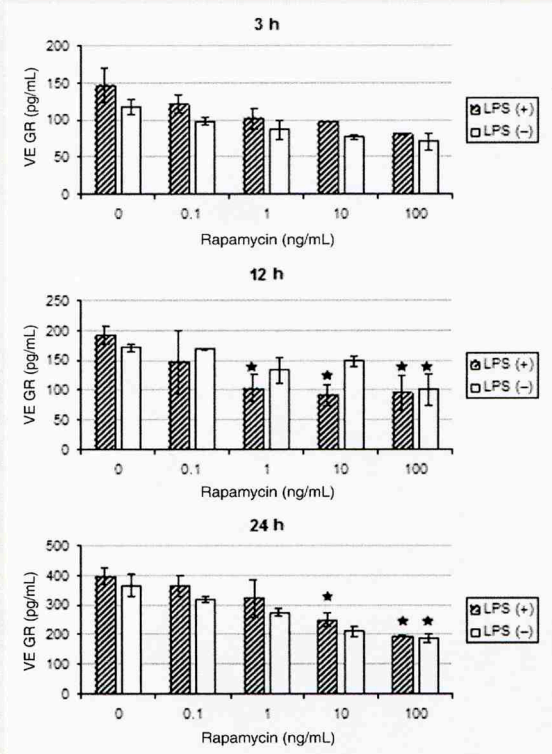

Figure 4 VEGF serum protein levels in THP-1 monocytes, at different rapamycin concentrations and at various time points, with or without LPS activation. Stars denote significant difference from baseline; VEGF, vascular endothelial growth factor; LPS, lipopolysachharide.

stent implantation and that they are related to in-stent neointimal hyperplasia.[2,13] The increase of VEGF gene expression that we found in these cells may reflect their activation after stent implantation, which could play an early role in regulating vascular wall response in the inflammatory processes following post-procedural wall injury and triggering pathophysiological mechanisms leading to restenosis.

Effects of SES on VEGF serum protein levels and VEGF gene expression in monocytes

According to our results, after SES implantation both VEGF protein serum level and VEGF gene expression in peripheral monocytes decreased. The latter decrease was positively correlated with the in-stent late luminal loss, suggesting that it could play an important part in the reduced restenosis rate associated with these stents.

Our *in vitro* results in the human monocyte-like myeloma cell line THP-1 showed that the cause of the decrease in VEGF gene expression in monocytes was the drug with which the stent was treated, rapamycin. Although our study did not assess the mode of action of rapamycin in reducing the genetic expression in monocytes, we can certainly say that the concentrations needed for this action are very low (1 ng/mL at 3 h and 0.1 ng/mL at 12 h) and far below toxic levels. This result, in combination with the fact that systemic levels of rapamycin within the first hours after SES implantation have been reported to be in the range of 1–2 ng/mL,[14] suggests that SES may have not only a local action but also a systemic effect on monocytes, explaining our *in vivo* results regarding the reduction of VEGF gene expression in peripheral monocytes.

Another finding of our *in vitro* study was that the reduction in VEGF gene expression caused by rapamycin in monocytes results in a significant reduction in their VEGF protein secretion. This effect must certainly play an important role in the decrease in VEGF serum protein levels that we found *in vivo*. However, we cannot rule out the possibility that the decrease in VEGF levels resulted from the action of rapamycin on other cells, or even from another action of rapamycin. Reports in previous studies that rapamycin can inhibit tumour growth by anti-angiogenic mechanisms and diminish the expression of VEGF by tumour cells reinforce the former hypothesis.[15] We know that various types of cells, including cardiomyocytes, vascular smooth muscle cells, as well as megakaryocytes, lymphocytes, and neutrophils, can secrete VEGF. On the other hand, previous studies have found that VEGF contributes to the pathophysiology of the inflammatory response after stent implantation and that SES reduces this response.[16] Such an action could also partly explain a reduction in VEGF expression.

The significant reduction in VEGF serum protein levels following SES implantation may not be related with the reduced restenosis rate in these stents compared with BMS, but might be related with the lower incidence of major adverse clinical events. This hypothesis is supported by the finding from previous studies that elevated VEGF serum levels were significantly associated with a worse clinical outcome in patients with acute myocardial infarction.[17]

Comparison with previous studies

To our knowledge there is only one study[18] that evaluated the behaviour of VEGF serum protein levels after angioplasty in humans. In this study, which included patients with stable coronary artery disease, VEGF protein levels did not change significantly. However, the VEGF serum protein levels were measured only 12 h after angioplasty and so might not have shown the changes that we observed at 1 month. In addition, the patient population differed from ours and it was not reported whether stents were used, or if so what kind. For these reasons the results may not be comparable with those of our own.

Study implications—Conclusions

Our study is the first in living humans to investigate the role of VEGF in neointimal growth after implantation of different kinds of stents. According to our results, both protein serum levels and gene expression in peripheral monocytes are changed following stent implantation. Furthermore, these changes in VEGF mRNA in peripheral monocytes are positively correlated with the in-stent late luminal loss, suggesting that they may play an important role in restenosis. This raises a novel perception of the mechanisms of

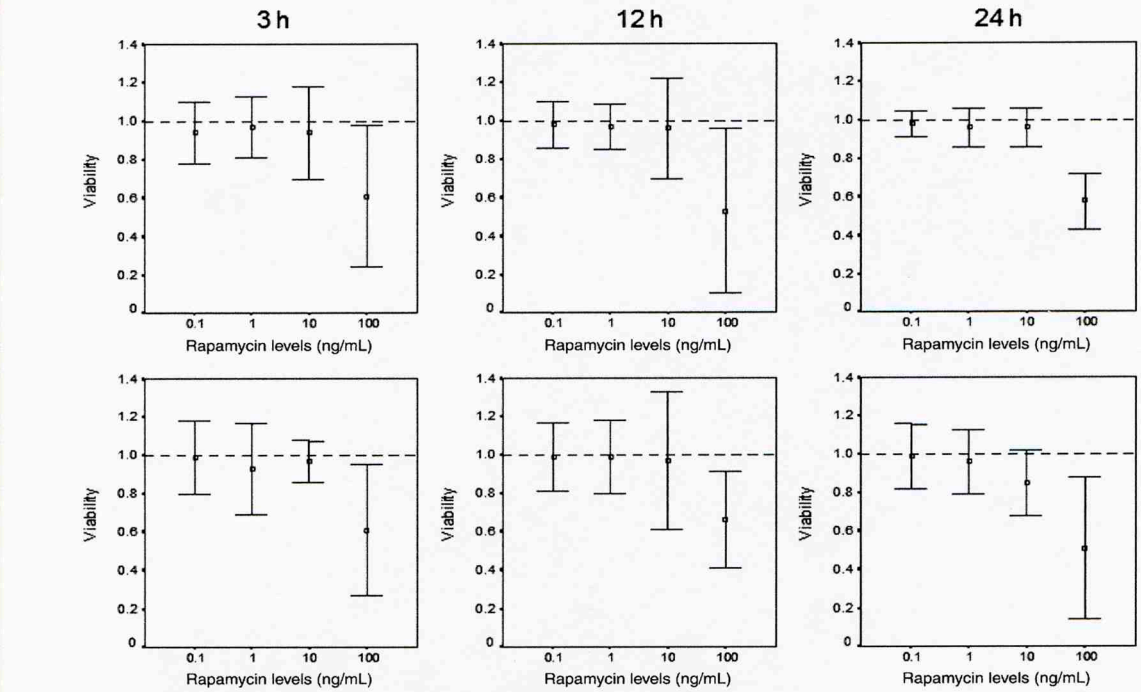

Figure 5 95% CI for cell viability at different rapamycin concentrations and various time points. Error bars that fall below one indicate significant change with respect to untreated cells. Upper row, LPS (−); lower row, LPS (+); CI, confidence interval; LPS, lipopolysachharide.

in-stent restenosis and might suggest another therapeutic target for the reduction of restenosis. SES down-regulates VEGF gene expression in peripheral monocytes, in contrast to BMS, which have the opposite effect; this may be another reason why SES shows a low incidence of restenosis in clinical practice. Further studies are needed to evaluate whether the action of SES in reducing VEGF protein levels has a beneficial effect on prognosis.

Limitations

We assessed serum protein levels of VEGF as well as VEGF gene expression in the peripheral monocytes since it was infeasible to study VEGF protein and mRNA in the region of the stent in humans. This certainly does not give a precise picture of what happens locally in the vicinity of the stent. Nevertheless, even this imperfect method was sufficient to detect a significant differential response between the two types of stent.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Acknowledgements

The authors would like to thank Dr. Christos Tsatsanis, Department of Clinical Chemistry-Biochemistry, Medical School, University of Crete, who kindly provided the human monocytic-like myeloma cell line THP-1.

Conflict of interest: none declared.

Funding

The study was funded by an award from the Hellenic Cardiological Society.

References

1. Schampaert E, Moses JW, Schofer J, Schluter M, Gershlick AH, Cohen EA, Palisaitis DA, Breithardt G, Donohoe DJ, Wang H, Popma JJ, Kuntz RE, Leon MB. SIRIUS, E- and C-SIRIUS Investigators. Sirolimus-Eluting Stents at two years: a pooled analysis of SIRIUS, E-SIRIUS, and C-SIRIUS with emphasis on late revascularizations and stent thromboses. *Am J Cardiol* 2006;**98**:36–41.
2. Fukuda D, Shimada K, Tanaka A, Kawarabayashi T, Toshiyama M, Toshikawa J. Circulating monocytes and in-stent neointima after coronary stent implantation. *J Am Coll Cardiol* 2004;**43**:18–23.
3. Gawaz M, Neumann FG, Ott I, May A, Rudiger S, Schomig A. Changes in membrane glycoproteins of circulating platelets after coronary stent implantation. *Heart* 1996;**76**:166–172.
4. Shibata M, Suzuki H, Nakatani M, Koba S, Geshi E, Katagiri T, Takeyama Y. The involvement of vascular endothelial growth factor and flt-1 in the process of neointimal proliferation in pig coronary arteries following stent implantation. *Histochem Cell Biol* 2001;**116**:471–481.

5. Cervinka P, Costa MA, Angiolillo DJ, Spacek R, Bystron M, Kvasnak M, Veselka J, Nanda H, Futamatsu H, Futamatsu K. Head-to-head comparison between sirolimus-eluting and paclitaxel-eluting stents in patients with complex coronary artery disease: an intravascular ultrasound study. *Catheter Cardiovasc Interv* 2006;**67**:846–851.
6. Berse B, Brown LF, Water LVD, Dvorak HF, Senger DR. Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. *Mol Biol Cell* 1992;**3**:211–220.
7. Panutsopulos D, Arvanitis DL, Tsatsanis C, Papalambros E, Sigala F, Spandidos DA. Expression of heregulin in human coronary atherosclerotic lesions. *J Vasc Res* 2005;**42**:463–474.
8. Tsatsanis C, Zacharioudaki V, Androulidaki A, Dermitzaki E, Charalampopoulos I, Minas V, Gravanis A, Margioris AN. Adiponectin induces TNF-α and IL-6 in macrophages and promotes tolerance to itself and other pro-inflammatory stimuli. *Biochem Biophys Res Commun* 2005;**335**:1254–1263.
9. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;**65**:55–63.
10. Risau W. Mechanisms of angiogenesis. *Nature* 1997;**386**:671–674.
11. Lazarous DF, Shou M, Scheinowitz M, Hodge E, Thirumurti V, Kitsiou AN, Stiber JA, Lobo AD, Hunsberger S, Guetta E, Epstein SE, Unger EF. Comparative effects of basic fibroblast growth factor and vascular endothelial growth factor on coronary collateral development and the arterial response to injury. *Circulation* 1996;**94**:1074–1082.
12. Van Belle E, Tio FO, Chen D, Maillard L, Chen D, Kearney M, Isner JM. Passivation of metallic stents after arterial gene transfer of ph VEGF 165 inhibits thrombus formation and intimal thickening. *J Am Coll Cardiol* 1997;**29**:1371–1379.
13. Rogers C, Welt FGP, Karnovsky MJ, Edelman ER. Monocyte recruitment and neointimal hyperplasia in rabbits: coupled inhibitory effects of heparin. *Arterioscler Thromb Vasc Biol* 1996;**16**:1312–1318.
14. Iakovou I, Sangiorgi GM, Stankovic G, Corvaja N, Vitrella G, Ferraro M, Colombo A. Results and follow-up after implantation of four or more Sirolimus-eluting stents in the same patients. *Catheter Cardiovasc Interv* 2005;**64**:436–439.
15. Kwon YS, Kim JC. Inhibition of corneal neovascularization by rapamycin. *Exp Mol Med* 2006;**38**:173–179.
16. Kochiadakis GE, Marketou ME, Arfanakis DA, Sfiridaki K, Skalidis EI, Igoumenidis NE, Hamilos MI, Kolyvaki S, Chlouverakis G, Kantidaki E, Castanas E, Vardas PE. Reduced systemic inflammatory response to implantation of sirolimus-eluting stents in patients with stable coronary artery disease. *Atherosclerosis* 2007;**194**:433–438.
17. Hojo Y, Ikeda U, Zhu Y, Okada M, Ueno S, Arakawa H, Fujikawa H, Katsuki T, Shimada K. Expression of vascular endothelial growth factor in patients with acute myocardial infarction. *J Am Coll Cardiol* 2000;**35**:968–973.
18. Banerjee S, Brilakis E, Zhang S, Roesle M, Lindsey J, Philips B, Blewett CG, Terada LS. Endothelial progenitor cell mobilization after percutaneous coronary intervention. *Atherosclerosis* 2006;**189**:70–75.