Inverse Association of Coronary Soluble Tumor Necrosis Factor-Related Apoptosis Inducing Ligand (sTRAIL) Levels to In-Stent Neointimal Hyperplasia

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For editorial comment see p. 94

Key Words

Apoptosis • sTRAIL • In-stent restenosis • Intravascular ultrasound • Neointima

Abstract

Objectives: Soluble tumor necrosis factor-related apoptosis inducing ligand (sTRAIL) has been shown to exert protective action against atherosclerosis. The aim of this study was to investigate potential associations of coronary sTRAIL levels with indices of in-stent neointimal hyperplasia. **Methods:** 67 patients who underwent percutaneous coronary intervention with drug-eluting stent were followed up at approximately 12 months with determination of coronary sTRAIL concentration, angiography and intravascular ultrasound evaluation of the stent sites. **Results:** Mean sTRAIL concentration was 72.2 ± 2.8 pg/ml. sTRAIL was negatively correlated to indices of neointimal hyperplasia and positively correlated to in-stent minimum lumen area ($p < 0.001$). Neointimal obstruction and maximal in-stent cross-sectional neointima burden in patients in the upper sTRAIL quartile

were 3.8 ± 1.2 and $12.6 \pm 3.3\%$, respectively, versus 14.0 ± 0.7 and $49.8 \pm 2.7\%$ in the lower quartile ($p < 0.001$ for both). sTRAIL levels were significantly lower in patients with binary restenosis (48.7 ± 3.0 vs. 75.2 ± 2.9 pg/ml; $p < 0.001$). In the multivariate analysis, sTRAIL was an independent predictor of neointimal hyperplasia. **Conclusion:** This study demonstrates a negative association of sTRAIL to in-stent neointima formation. The potential pathophysiologic substrate of this effect implicates modulation of apoptosis in various cell types. These observations should prompt further evaluation of the link between sTRAIL and in-stent restenosis.

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Introduction

Neointimal hyperplasia is known to play a dominant role in the pathogenesis of in-stent restenosis following percutaneous coronary interventions (PCI) with stent implantation [1]. There seems to be a delicate balance between

cell proliferation and apoptosis in vascular smooth muscle cells, with apoptosis signaling molecules being implicated in the regulation of vascular cell proliferation and, thus, linked to the processes leading to atherosclerotic plaque development [2]. Apoptotic indices have been linked to the degree of in-stent restenosis in vivo in patients following PCI [3]. The tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) is a protein, member of the TNF ligand superfamily, which can be secreted as a vesicle-associated form [4] or a soluble form [5, 6]. The exact mechanisms underlying the role of soluble TRAIL (sTRAIL) in the process of coronary atherosclerosis are uncertain, since downstream signaling, following binding of sTRAIL to receptors on human vascular muscle and endothelial cells, has been shown to lead both to apoptosis [7] and promotion of cell survival [8, 9]. However, most evidence from experimental [10] and clinical [11] studies indicate that sTRAIL may play a protective role against atherosclerosis, although there is also evidence to the contrary [11].

The principal aim of the present study was to measure coronary sTRAIL levels in patients with previous PCI and investigate potential associations with intravascular ultrasound (IVUS)-derived parameters of in-stent neointimal hyperplasia.

Methods

Population

This was a cross-sectional, one-group study. Patients with previous PCI with drug-eluting stent(s) underwent follow-up angiography with IVUS evaluation of all implanted stents 10–14 months after stent implantation. Exclusion criteria were: acute coronary syndrome, calculated creatinine clearance <30 ml/h/1.73 m², left ventricular ejection fraction <40%, coronary anatomy unfavorable for IVUS study (excessive tortuosity, steep take-off angle of the target vessel), discontinuation of both antiplatelet agents (aspirin and clopidogrel) or statin during the time elapsed from the index PCI. The angiogram-IVUS study was performed regardless of symptoms after obtaining written informed consent from all patients. The study was approved by the institutional review board and was implemented according to the provisions of the Declaration of Helsinki.

Procedures

Left cardiac catheterization was performed through the transradial or the transfemoral route. Immediately after cannulation of each coronary artery (left main and right coronary artery) blood was drawn through the angiography catheter. sTRAIL was measured using a commercially available enzyme immunoassay kit (R&D Systems, Minneapolis, Minn., USA) according to the manufacturer's instructions in a certified research laboratory. The assay sensitivity is 0.57–7.87 pg/ml (minimum detectable concentration 2.86 pg/ml).

Standard coronary angiography followed. IVUS was used to study all implanted stents. After intracoronary administration of 0.3 mg nitroglycerin, a digital IVUS catheter (Eagle Eye® Gold, Volcano Co., Rancho Cordova, Calif., USA) was introduced into the target vessel and a pullback was performed through the implanted stent, with a motorized automatic pullback system (Track Back® II, Volcano Co.) at a constant speed of 0.5 mm/s.

Captured data were analyzed off-line on an s5™ Imaging System workstation (Volcano Co.). Each recorded pullback loop was inspected by formally trained IVUS operators (certified by official IVUS-training teaching centers), who made manual corrections to the automated border delineation applied by the system software. Volumetric plaque data were then automatically calculated. Neointima volume was calculated as stent minus lumen volume and divided by the stent length in millimeters to account for different stent lengths (volume index – VI). Percent neointimal obstruction was defined as neointimal volume divided by stent volume. Cross-sectional neointimal burden (CNB, %) was defined as the neointimal area divided by stent area. Maximal in-stent CNB equal to or greater than 60% was considered as IVUS-defined binary in-stent restenosis [12]. In-stent minimum lumen area (MLA) was also measured and recorded. A subset of the pullbacks (approximately 20%) was analyzed twice, unbeknownst to the reviewers. The intra-observer correlation index was 0.90 for lumen measurements and 0.87 for plaque volumes.

Statistical Analysis

Data analysis was performed per implanted stent. Each artery where a pullback was performed was matched to the sTRAIL concentration measured in the blood drawn from the left main (for stents in the left anterior descending and left circumflex artery) or the right coronary artery. Continuous variables were expressed as mean ± standard error of the mean (SEM), unless otherwise indicated. If their distribution deviated significantly from normal, nonparametric tests were applied. Categorical variables were expressed as percentages and counts and compared using the χ^2 test or Fisher's exact test. Multivariate analysis of variance (MANOVA) was applied to test associations to in-stent neointimal hyperplasia indices (neointima VI, neointimal obstruction, MLA and CNB). The variables which were univariately associated to these indices were entered as independent parameters in the model. Predictors of binary restenosis were tested using binary logistic regression analysis. IBM® SPSS® Statistics 19.0 software package was used (SPSS Inc., Chicago, Ill., USA). Two-sided p values <0.05 were considered to be significant.

Results

One hundred and three stented arterial segments were studied in 67 patients (44 male; aged 65.1 ± 0.9), satisfying the criteria outlined in Methods. The basic characteristics are summarized in table 1.

Mean coronary sTRAIL concentration was 72.2 ± 2.8 pg/ml. It did not show significant variation with gender ($p = 0.856$), but was significantly, although weakly, correlated to age ($r = -0.38$, $p < 0.001$) and left ventricular

ejection fraction ($r = 0.52$, $p < 0.001$). sTRAIL was lower in diabetics (60.5 ± 2.6 vs. 86.1 ± 4.5 pg/ml; $p < 0.001$). Current smoking, hypertension and dyslipidemia were not significantly associated with sTRAIL levels.

In-Stent Neointimal Hyperplasia and Restenosis Indices

In-stent neointima VI, neointimal obstruction, maximal in-stent CNB and in-stent MLA are summarized in table 1. Exploration of potential univariate associations of these indices to basic demographic and risk characteristics revealed the following significant relations. Neointimal VI was higher in men than women (0.9 ± 0.1 vs. 0.6 ± 0.1 mm³; $p = 0.036$), while the other three indices were not correlated to gender. Diabetes was a significant factor for all four indices, with neointima VI (1.0 ± 0.1 vs. 0.7 ± 0.1 mm³/mm; $p = 0.030$), neointima obstruction (10.0 vs. 6.4% ; $p = 0.002$) and CNB (40 ± 3 vs. $21 \pm 3\%$; $p < 0.001$) being higher in diabetics, while MLA was lower in diabetic patients (5.0 ± 0.1 vs. 5.7 ± 0.1 mm²; $p < 0.001$). Stent type was, as expected, significantly associated with neointima formation. Neointima VI, neointimal obstruction and maximal in-stent CNB were lower with sirolimus-eluting stents versus paclitaxel (0.3 ± 0.0 vs. 1.4 ± 0.1 mm³/mm, 3.5 ± 0.3 vs. $13.5 \pm 0.6\%$, 18 ± 2.5 vs. $46 \pm 2.0\%$, respectively; $p < 0.001$ for all). Correspondingly, MLA was higher with sirolimus-eluting stents (5.8 ± 0.1 vs. 4.8 ± 0.1 mm²; $p < 0.001$); of note, sTRAIL concentration was also higher in patients with sirolimus-eluting stents (89.3 ± 3.7 vs. 54.0 ± 2.3 pg/ml; $p < 0.001$). In addition, age was positively correlated to neointima VI ($p = 0.002$) and neointimal obstruction ($p = 0.022$) and inversely correlated to MLA ($p = 0.003$; Spearman's rho 0.30, 0.23 and -0.30 , respectively). Left ventricular ejection fraction was inversely correlated to neointima VI ($p < 0.001$), neointimal obstruction ($p < 0.001$) and maximal in-stent CNB ($p < 0.001$), and positively correlated to MLA ($p < 0.001$; Spearman's rho -0.66 , -0.66 , -0.68 and 0.64 , respectively). Stent nominal diameter was also an important univariate factor, positively correlated to neointima VI ($p < 0.001$), neointimal obstruction ($p < 0.001$) and maximal in-stent CNB ($p < 0.001$), and inversely correlated to MLA ($p < 0.001$; Spearman's rho 0.55, 0.56, 0.57 and -0.37 , respectively).

Stent nominal diameter and stent length, as well as left ventricular ejection fraction were associated to the presence of binary restenosis ($p < 0.01$ for all). The proportion of men among patients with restenosis was higher (83%), compared to 64% among those without binary restenosis, without reaching statistical significance ($p = 0.214$). Dia-

Table 1. Basic population characteristics and coronary imaging findings

	Patients (n = 67)
Male gender	44 (66)
Age, years	65.1 ± 0.9
Diabetes mellitus	29 (43)
Hypertension	34 (51)
Dyslipidemia	34 (51)
Current smoking	27 (40)
LVEF, %	57 ± 0.6
	Stented arterial segments (n = 103)
Coronary artery	
Left anterior descending artery	44 (66)
Left circumflex artery	10 (15)
Right coronary artery	13 (19)
Drug-eluting stent type	
Sirolimus	53 (52)
Paclitaxel	50 (49)
Stent length, mm	27.6 ± 0.4
Stent nominal diameter, mm	3.3 ± 0.04
Neointima VI, mm ³ /mm	0.8 ± 0.06
Minimum lumen area, mm ²	5.3 ± 0.09
Neointima obstruction, %	8.3 ± 0.6
Cross-sectional neointima burden, %	31 ± 2.1
sTRAIL, pg/ml	72 ± 2.8
Binary restenosis	12 (11.7)

Continuous variables are expressed as mean $\pm$ SEM and categorical variables as count with the percentage in parentheses.

LVEF = Left ventricular ejection fraction.

betes, on the other hand, was significantly more frequent among those with restenosis ($p < 0.001$). Paclitaxel-eluting stent, although associated with more marked in-stent neointima formation, as described in the previous paragraph, was not significantly correlated to binary restenosis ($p = 0.470$).

Coronary sTRAIL appeared to be protective against in-stent neointimal growth, exhibiting negative correlation to indices of neointimal hyperplasia and positive correlation to MLA ($p < 0.001$ for all; fig. 1). The associations between sTRAIL and neointimal obstruction as well as between sTRAIL and in-stent CNB exhibited a similar significant trend (neointimal obstruction and maximal in-stent CNB of patients in the upper sTRAIL quartile were 3.8 ± 1.2 and $12.6 \pm 3.3\%$, respectively, vs. 14.0 ± 0.7 and $49.8 \pm 2.7\%$ in the lower sTRAIL quartile;

Fig. 1. Mean values (with 95% confidence intervals) of in-stent neointima VI (a) and MLA (b) in each quartile of sTRAIL concentration.

Table 2. Results of multivariate analysis of variance test (Wilks' lambda)

Variables	Wilks' lambda	P
Age	0.91	0.071
Gender	0.91	0.069
Stent diameter	0.71	<0.001
LVEF	0.94	0.256
sTRAIL	0.81	<0.001
Diabetes mellitus	0.86	<0.009
Stent type (PES or SES)	0.52	<0.001

All variables univariately associated to in-stent neointima volume index, neointimal obstruction, in-stent minimum lumen area and in-stent cross-sectional neointima burden (dependent variables) were entered as independent variables.

LVEF = Left ventricular ejection fraction; PES = paclitaxel-eluting stent; SES = sirolimus-eluting stent.

$p < 0.001$ for all). In regard to in-stent binary restenosis, sTRAIL levels were significantly lower in patients with restenosis (48.7 ± 3.0 vs. 75.2 ± 2.9 pg/ml; $p < 0.001$).

Multivariate Analysis

In the MANOVA performed to test for independent associations with neointimal hyperplasia indices, neointima VI, neointimal obstruction, MLA and CNB were entered as dependent variables and age, gender, left ventricular ejection fraction, stent type, stent diameter, dia-

betes and sTRAIL level were the independent variables (univariate correlates of neointima formation). The results (table 2) clearly denote that the stent type and diameter, diabetes mellitus and sTRAIL levels were independent predictors of the four in-stent neointimal hyperplasia parameters.

Discussion

The findings of the present study suggest a protective role of coronary artery sTRAIL against in-stent neointimal hyperplasia, as it was shown that coronary artery sTRAIL levels are inversely correlated to IVUS-derived markers of neointima formation. This was true for all four measured indices of in-stent neointimal hyperplasia. sTRAIL levels were found to be independently correlated to these parameters in the multivariate analysis, which suggests a link – in fact a strong negative association – between sTRAIL and in-stent neointima formation.

These results are in keeping with previous experimental and clinical reports of an inverse association of sTRAIL levels to atherosclerotic disease, both in experimental and clinical studies [10, 13–16]. However, some of these studies have yielded contradicting results in regard to specific aspects of the role of sTRAIL in atherosclerotic processes. In particular, while Watt et al. [10] showed a link between TRAIL deficiency and markedly increased atheromatous burden, they did not find significant differences in plaque composition. On the other hand, Di

Bartolo et al. [16] observed clear signs of increased plaque vulnerability in TRAIL-deficient mice: thinner fibrous cap, more extensive necrotic cores and macrophage infiltration. The differences might be attributed to different diet fat contents between the two studies. These inconsistencies between observations underscore the multiplicity of interactions between TRAIL and various inborn and environmental factors, which render the investigation of its role in atheromatosis an onerous task, subject to several potential influences and confounders. Our group has shown that sTRAIL levels are inversely associated to IVUS-defined coronary atheromatosis burden, as well as to the quantity of necrolipidic plaque content (using IVUS-virtual histology) [17]. TRAIL is a member of the TNF superfamily [18, 19]. sTRAIL acts on various cellular constituents of the vascular wall, including the endothelium and smooth muscle cells [13], through the Akt and extracellular signal-regulated kinase pathways [8]. In addition, it promotes endothelial production of nitric oxide and prostanoid derivatives [20] and has been shown to be reduced in patients with acute coronary syndromes [13]. Accordingly, Secchiero et al. [21] have shown that sTRAIL is reduced in patients with acute myocardial infarction.

Of note, although existing evidence regarding the role of sTRAIL in atherosclerosis pertains mainly to primary atherosclerotic processes, a relationship with restenosis after PCI has been suggested in an interesting report by Satoh et al. [22], where sTRAIL was found to be negatively associated with angiographic restenosis: sTRAIL levels after PCI were significantly increased in the non-restenosis group and sTRAIL levels were related to soluble vascular cell adhesion molecule-1 and soluble E-selectin. In addition, Watt et al. [10] showed that TRAIL-deficient mice had higher levels of smooth muscle cell activation and larger early atherosclerotic lesions compared to non-TRAIL-deficient mice. Furthermore, sTRAIL inhibits mediators of the Akt pathway [8, 10], which has been implicated in vascular restenosis after balloon injury and stenting [23]. On the other hand, in a contradicting report by Chan et al. [11], increased TRAIL expression has been linked to vascular smooth muscle cell proliferation and neointima formation after arterial injury. However, the weight of evidence in various settings of atherosclerotic artery disease, as already mentioned, points toward a protective effect of sTRAIL against atherosclerosis [10, 13–17, 21]. This theoretical framework is substantiated in vivo by our present observation, that coronary artery sTRAIL is inversely correlated to several indices of in-stent neointima hyperplasia.

Study Limitations

IVUS image analysis was performed by certified IVUS operators, who interpret a substantial number of IVUS studies on a monthly basis, but not in a separate core laboratory. This could be a source of bias, although it should be noted that IVUS image interpretation was performed in a blinded manner. The invasive nature of the present study (coronary angiography and IVUS) precluded the inclusion of a larger number of subjects, which made the assessment of clinical outcomes, such as ischemia-driven revascularization of the target lesion, statistically implausible.

Conclusion

The present study demonstrates an inverse association of sTRAIL to in-stent neointima formation. The results imply that this association may be biologically relevant in vivo, although its true clinical significance is unclear. We believe that the presented observations should prompt further evaluation of the link between sTRAIL and in-stent restenosis, which may deepen our knowledge regarding the relation between apoptotic processes and in-stent neointima formation.

Conflict of Interest

None.

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