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Association of serum TRAIL level with coronary artery disease

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ABSTRACT

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF ligand family, and is able to induce apoptosis in tumor cells. Emerging experimental findings suggest the involvement of TRAIL in vascular biology and atherosclerosis. However, little is known concerning the role of TRAIL in atherosclerosis in humans. We therefore examined whether serum TRAIL levels are associated with coronary artery disease (CAD). Serum TRAIL levels were measured by ELISA in 285 patients who underwent coronary angiography. Serum TRAIL level was significantly lower in patients with CAD (659.2 ± 176.6 pg/ml) than in those without CAD (732.3 ± 187.9 pg/ml, $p = 0.0016$). Next, the number of diseased vessels was used to represent the severity of CAD. Serum TRAIL levels were inversely associated with the severity of CAD (p for trend = 0.0005). In particular, TRAIL levels in patients with severe 3-vessel disease were significantly lower than those in subjects without CAD (602.9 ± 150.0, 732.3 ± 187.9 pg/ml, respectively; $p < 0.05$). Multivariable logistic regression analysis revealed that serum TRAIL levels were significantly associated with the presence of CAD (odds ratio, 0.68; 95% confidence interval 0.51-0.90; $p = 0.006$). Serum TRAIL levels were inversely associated with the advanced CAD, suggesting that TRAIL may be useful as a biomarker of CAD severity.

Introduction

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) belongs to the TNF ligand superfamily. TRAIL binds to five different receptors found on a variety of types of cells. Of these receptors, two, DR4 (TRAIL-R1) and DR5 (TRAIL-R2), contain a cytoplasmic death domain and trigger TRAIL- induced apoptosis. Two decoy receptors lacking a functional death domain, DcR1 (TRAIL-R3) and DcR2 (TRAIL-R4), compete with DR4 and DR5 for TRAIL binding, possibly antagonizing apoptotic signaling. In addition, osteoprotegerin is known to be a fifth soluble decoy receptor [1,2].

Atherosclerosis is a chronic arterial disease that develops through multiple steps. In the progression of atherosclerotic plaque, inflammatory and immune responses caused by complex interactions between modified lipoproteins and various cells that constitute the arterial wall are involved. Endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) are principal cellular components in normal arterial wall, while inflammatory cells such as activated T lymphocytes and macrophages play crucial roles in atherogenesis [3]. Interestingly, TRAIL and its receptors are expressed in both physiological and pathological arterial wall [4–7]. Expression of TRAIL has been confirmed in both resident cells (ECs, VSMCs) and infiltrating inflammatory cells (monocytes/macrophages, T lymphocytes) within the arterial wall [4–6]. As expected, TRAIL can induce apoptosis in ECs [8,9], VSMCs [10], lymphocytes [11], and macrophages [12] *in vitro*. On the other hand, it has been reported that TRAIL can stimulate anti-apoptotic proliferation and/or migration in ECs [13] and VSMCs [14,15] under some conditions. Moreover, TRAIL promoted nitric oxide (NO) synthesis in ECs [16] and inhibited pro-inflammatory cytokine-induced leukocyte adhesion to ECs [17]. Taken together, these findings suggest the multifunctional involvement of TRAIL in atherosclerosis and other aspects of vascular homeostasis beyond its apoptosis-inducing effect, although its precise roles in the vasculature as a whole are complex and remain unclear.

Recent *in vivo* findings have revealed direct effects of TRAIL on atherosclerotic lesions. Systemic administration of recombinant TRAIL protected against progression of atherosclerosis in apolipoprotein-E deficient diabetic mice, mainly via induction of apoptosis of infiltrating macrophages [18]. In humans, only two studies have yet been performed on the function of TRAIL in atherogenesis. One clinical study reported that serum TRAIL levels were significantly reduced in patients with acute coronary syndrome (ACS) compared with healthy subjects [19]. Another study reported that serum TRAIL levels tended to be lower in patients with coronary artery disease (CAD) than in subjects without CAD, though not to a significant extent [20]. These

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findings suggest that TRAIL may contribute to atherogenesis in humans, as well. In this study, we assessed the severity of CAD by coronary angiography and examined whether serum TRAIL levels are associated with CAD.

Materials and methods

Patients

This study involved 285 patients who underwent elective diagnostic coronary angiography [21]. Diagnostic angiography was indicated for patients with typical symptom of effort angina or with atypical chest pain and positive signs of exercise electrocardiography test. Positive signs of stress electrocardiography were considered as an abnormal ST segment response with horizontal or down-sloping depression of > 1 mm, an abnormal ST segment elevation > 1 mm in non-Q wave leads, onset of severe ventricular arrythmia, development of left bundle branch block, progression of heart block to second/third degree, cardiac arrest. Patients with acute coronary syndrome or overt renal dysfunction (creatinine level > 1.2 mg/dl (106 μmol/l)) were excluded. However, patients with previous histories of myocardial infarction, stroke, and peripheral vascular disease were included. At the time of physical examination, blood pressure, body mass index (BMI), and hematological and biochemical profiles were determined. Age and history of cigarette use were assessed through an interview preceding the physical examination. Written informed consent was obtained from all patients. The local ethics committee of Mitsui Memorial Hospital approved the study protocol.

One hundred twenty-eight patients were receiving an anti-anginal drug (Isosorbide dinitrate). Diabetes was considered present if a patient was being treated with insulin or oral agents or had a fasting glucose level of ≥126 mg/dl (7.0 mmol/l). Forty-eight subjects were receiving anti-diabetic drug treatment, in the form of either insulin injection (9 subjects) or sulfonylureas (39 subjects). Hypertension was defined by systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 mmHg, current use of antihypertensive treatment, or a combination of these. Two hundred sixteen of the patients were receiving antihypertensive treatment, with calcium channel blockers (109 subjects), angiotensin-converting enzyme inhibitors (13 subjects), β-blockers (8 subjects), diuretics (3 subjects), or a combination of these (84 subjects). Hypercholesterolemia was defined as total cholesterol level of >220 mg/dl (5.69 mmol/l), current use of lipid-lowering treatment, or both. Seventy subjects were receiving lipid-lowering drugs (HMG-CoA reductase inhibitors).

Coronary Angiography

Quantitative coronary angiography (QCA) was performed, using an automated edge detection system CMS (MEDIS, Leiden, The Netherlands) by experienced cardiologists who were unaware of clinical and biological data. Significant coronary stenosis was defined as ≥50% diameter narrowing based on QCA measurement. Variability on repeat measurements of percent diameter stenosis was 2.7%. The severity of CAD was represented as the number of diseased vessels [21].

Serum TRAIL Measurement

Fasting serum samples were collected and stored at −80°C until use. Serum TRAIL levels were determined using a sandwich enzyme-linked immunosorbent assay (BioSource International Inc., Camarillo, California) in a blinded fashion and in random order. All samples were measured in duplicate and averaged. The limit of detection of this assay system was 20 pg/ml, and the intra- and interassay CV values were <4.8% and <6.0%, respectively.

Statistical analysis

All values are presented as the mean ± SD or the median (interquartile range). To compare baseline characteristics among the four groups (CAD) by extent of coronary angiographic disease, one-way analyses of variance or Kruskal-Wallis tests or χ^2 tests were performed. To evaluate the relationships between TRAIL and various clinical parameters, simple linear regression analyses were performed. Spearman's rank correlation was used to determine correlations between TRAIL and fasting glucose, triglyceride, high-sensitivity CRP. The unpaired Student's t- test for continuous variables or χ^2 tests for categorical variables were used to compare various clinical parameters including TRAIL levels between the two groups with or without CAD. Differences in TRAIL levels among categories by CAD severity were examined by one-way ANOVA and Dunnett's test for multiple comparisons. We performed logistic regression analyses with stepwise variable selection. Only factors with $p<0.10$ on univariable analyses were included in this multivariable logistic regression. Odds ratios (ORs) are presented with 95% confidence intervals (CIs). P values<0.05 were considered significant. All p values were two-tailed.

Results

The study group included 224 men and 61 women. The clinical characteristics of the study group are shown in Table 1. The prevalence of cardiac risk factors was 76 % for hypertension, 38 % for diabetes, 29 % for hypercholesterolemia, and 39% for current smoking. On the basis of angiographic analysis, 194 (68 %) of the subjects were characterized as having significant stenosis. We found 86 patients with one-vessel disease (1-VD), 53 patients with two-vessel disease (2-VD), 55 patients with three-vessel disease (3-VD), and 91 patients with no diseased vessel or luminal irregularities (No-VD).

To examine the relationship between circulating TRAIL and CAD, we measured serum TRAIL levels in all 285 subjects. The mean serum level of TRAIL was 682.5 ± 183.2 (pg/ml), with a range of 238.5 to 1377.0, and did not differ significantly between men and women (673.2 ± 178.5 vs. 716.7 ± 197.2 pg/ml, respectively). As previously reported [20], serum TRAIL level was correlated with age ($r=-0.135$, $p=0.023$). However, there was no correlation between TRAIL level and other possible CAD risk factors including body mass index ($r=0.103$, $p=0.082$), systolic blood pressure ($r=0.008$, $p=0.900$), diastolic blood pressure ($r=0.070$, $p=0.245$), fasting glucose ($\rho=0.081$, $p=0.191$), triglyceride ($\rho=0.082$, $p=0.193$), and HDL ($r=0.074$, $p=0.242$), except total cholesterol ($r=0.185$, $p=0.003$). No significant relationships were observed between TRAIL levels and high-sensitivity CRP ($\rho=0.056$, $p=0.354$) in this study.

Comparison of various clinical parameters including TRAIL levels between the two groups with or without CAD was carried out (Table 2). Serum TRAIL level was significantly lower in patients with CAD (659.2 ± 176.6 pg/ml) than in those without CAD (732.3 ± 187.9 pg/ml, $p=0.0016$). Furthermore, TRAIL level was inversely associated with the severity of CAD (p for trend = 0.0005) (No-VD: 732.3 ± 187.9, 1-VD: 687.0 ± 196.3, 2-VD: 672.5 ± 156.9, 3-VD: 602.9 ± 150.0 pg/ml) (Fig. 1). TRAIL levels in patients with severe 3-VD were significantly decreased compared with those in subjects with No-VD ($p<0.05$).

To identify factors affecting the presence of CAD, logistic regression analyses with stepwise variable selection were performed. Only variables with $P<0.10$ on univariable analyses (Table 2) were included. A 1-SD increase in serum TRAIL concentration was associated with an odds ratio of 0.68 (95% CI, 0.51 to 0.90; $P=0.006$) for having a CAD (Table 3). Hypertension and male sex were each positively associated with the presence of CAD. Intriguingly, the use of anti-diabetic drugs and statins was positive contributor for having a CAD. However, the use of drugs for metabolic disorders may express a long lasting and previous exacerbation of these disorders, though such drug intervention may superficially improve actual metabolic parameters at