Soluble E-Selectin in Essential Hypertension: A Correlate of Vascular Structural Changes

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Background: Increased expression of the endothelial leukocyte adhesion molecule E-selectin is implicated in vascular disease and may accompany the development of hypertension. We evaluated plasma soluble (s) E-selectin to assess its relationship with endothelium-dependent and endothelium-independent vasodilation in patients with hypertension.

Methods: Thirty-one previously untreated and uncomplicated essential hypertensive patients were compared with 16 normotensive controls for changes in forearm blood flow (by strain-gauge plethysmography) in response to brachial artery infusion of the endothelium-dependent vasodilator acetylcholine, and of the endothelium-independent vasodilator sodium nitroprusside. As an index of structural changes, minimal forearm vascular resistances were calculated as the ratio between maximal vasodilation after 13 min of ischemia and mean blood pressure.

Results: Responses to acetylcholine were significantly lower and minimal forearm vascular resistances higher in hypertensives versus controls, whereas responses to nitroprusside were comparable. Baseline sE-selectin concentrations were (mean ± SEM) 37.4 ± 1.8 ng/mL in hypertensives and 27.8 ± 0.7 ng/mL in normotensives ($P < .001$). In essential hypertensive patients, a significant ($P < .01$) correlation with the response to nitroprusside ($r = -0.47$) was found, but not with the response to acetylcholine or minimal forearm vascular resistances. sE-selectin was also positively correlated with age and LDL cholesterol. At multivariate analysis, sE-selectin remained significantly correlated with nitroprusside responses and LDL cholesterol.

Conclusions: In patients with essential hypertension, plasma levels of sE-selectin are higher than in normotensive controls and mostly related to structural vascular changes. Am J Hypertens 2001;14:259–266 © 2001 American Journal of Hypertension, Ltd.

Key Words: Soluble adhesion molecules, E-selectin, essential hypertension, endothelial dysfunction, endothelin activation vascular structural changes.

Structural or functional changes in vascular endothelium are involved in the early development of vascular disease.[1,2] Not only the development of clinically overt atherosclerosis,[3] but also the mere presence of risk factors,[4,5] has been associated with changes in endothelial function. Such changes have been classically assessed by the vasodilatory response to intra-arterial acetylcholine.[1,6] In particular, patients with essential hypertension have been shown to have an impaired endothelium-dependent vasodilation.[7,8]

Biohumoral markers of endothelial integrity or function, including von Willebrand factor (vWF), plasminogen activator inhibitor-type 1 (PAI-1), and microalbuminuria, have also been reported elevated in essential hypertension. Soluble (s) endothelial leukocyte adhesion molecules have been recently proposed as new markers of endothelial function. Such molecules include E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1). In vascular diseases, E-selectin, which is an endothelium-specific molecule,[2,9] has been reported elevated in septic shock,[10] congestive heart failure,[11] coronary and carotid artery disease,[12] unstable an-

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Table 1. Clinical characteristics of patients studied (values are mean ± SEM)

Parameter	Normotensive Control Subjects (n = 16)	Essential Hypertensive Patients (n = 31)	P (unpaired t test)
Age (y)	43.9 ± 3.9	47.5 ± 1.6	NS
Gender (male/female)	10/6	18/13	NS
Smokers (yes/no)	5/11	10/21	NS
Systolic blood pressure (mm Hg)	119.9 ± 2.4	157.4 ± 2.1	<.001
Diastolic blood pressure (mm Hg)	78.3 ± 1.4	103.0 ± 1.1	<.001
Body mass index (kg/m^3)	24.6 ± 0.6	25.1 ± 0.5	NS
Glucose (mg/dL)	90.6 ± 3.2	93.9 ± 3.5	NS
Total cholesterol (mmol/L)	5.20 ± 0.13	5.45 ± 0.16	NS
HDL cholesterol (mmol/L)	1.22 ± 0.10	1.19 ± 0.05	NS
LDL cholesterol (mmol/L)	2.98 ± 0.09	3.25 ± 0.12	NS
Triglycerides (mg/dL)	163.2 ± 2.3	157.4 ± 2.1	NS
Serum creatinine (mg/dL)	0.9 ± 0.02	0.95 ± 0.02	NS
Creatinine clearance (mL/min)	110.6 ± 3.3	106.9 ± 3.4	NS
Left ventricular mass index (g/m^3)	90.4 ± 2.2	116.7 ± 3.1	<.001

SEM = standard error of the mean; NS = not significant; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

gina,[13] acute stroke,[14] and obese subjects,[15] and predicting restenosis in patients with intermittent claudication.[16] In essential hypertension, increased levels of E-selectin in a small number of patients compared to normotensive controls have been reported.[17,18] This has been subsequently confirmed in some,[19–21] but not all similar investigations.[22] Some investigations found elevations of sE-selectin only in subsets of patients with essential hypertension, namely those with impaired glucose tolerance[21] or salt-sensitive hypertension.[23]

We tested the hypothesis that a state of endothelial "activation," as assessed by levels of sE-selectin, is related to endothelial "dysfunction," as assessed by the vasodilatory response to acetylcholine, in essential hypertension. Alternatively, elevated levels of E-selectin might be related to endothelium-independent vascular responses, as assessed by the responses to nitroprusside or to an index of vascular structural changes, the forearm minimal vascular resistances. To this purpose, we determined plasma levels of sE-selectin in a population of untreated patients with essential hypertension and in a matched population of healthy controls, and sought to determine whether these levels are related to functional or structural vascular changes in this disease.

Methods

Patients

We studied 31 relatively young and otherwise healthy patients with recently diagnosed essential hypertension. Age was (mean ± SEM) 47.5 ± 1.6 years. Eighteen subjects were men and 13 women (Table 1). They were screened from an original group of 75 patients with new onset (<12 months) essential hypertension, recruited—with the collaboration of general practitioners—among subjects used to have their blood pressure (BP) measured at least once every 6 months. All subjects were characterized by the presence of a positive family history of essential hypertension and the detection of a supine arterial BP consistently >140/90 mm Hg. This measurement was obtained weekly at least three times by a mercury sphygmomanometer after 10 min of rest. Secondary forms of hypertension were ruled out by biohumoral, renal ultrasound, and, when indicated, by computerized tomography examinations or nuclear magnetic resonance angiography.

Baseline BP values were 157.4 ± 2.1/103.0 ± 1.1 mm Hg for systolic and diastolic BP, respectively.

We excluded subjects with total cholesterol >6.7 mmol/L, diabetes mellitus, serum creatinine ≥1.2 mg/dL, body mass index ≥27 kg/m^2, and major ongoing diseases. Patients also were free of major valvular or coronary heart disease, extracranial cerebral, or peripheral vascular disease. Of the original group screened, 44 subjects were excluded because of the presence of a secondary form of hypertension (n = 12), diabetes (n = 8), coronary heart disease (n = 9), or severe hypercholesterolemia (n = 15).

Sixteen healthy, sedentary, nonobese, sex- and age-matched subjects served as controls. They were not taking any drugs, with normal physical examination, normal routine blood and urine tests, normal BP, and normal electrocardiogram, abdominal ultrasounds, and ankle/arm pressure index. Age was 43.9 ± 3.9 years.

Hypertensives and normotensives were comparable as to all demographic and metabolic parameters investigated, and were only different for values of BP and left ventricular mass index (Table 1).

According to institutional guidelines, the protocol was approved by the local institutional review committee, all patients were informed of the investigational nature of the study, and gave written informed consent.

Baseline demographic, metabolic, and hemodynamic characteristics of recruited patients are summarized in Table 1.

Experimental Procedures

All forearm blood flow studies were performed at 8.00 AM after an overnight fast with the subjects lying supine in a quiet air-conditioned room (22°C to 24°C). A 21-gauge polyethylene cannula (Abbott, Sligo, Ireland) was inserted into the right brachial artery under local anesthesia (2% lidocaine), and connected through stopcocks to a pressure transducer (model MS20; Electromedics, Englewood, CO) for brachial artery systolic, diastolic, mean BP (1/3 pulse pressure + diastolic pressure), and heart rate monitoring (model VSM1; Physiocontrol, Redmond, WA), and for intra-arterial infusions. Forearm blood flow was measured in both forearms by strain-gauge venous plethysmography (Loosco, GL Loos, Amsterdam, The Netherlands).[24] Circulation to the hand was excluded 1 min before each forearm blood flow measurement by inflating a pediatric sphygmomanometric cuff around the wrist at suprasystolic BP. Details on the sensitivity and reproducibility of the method as performed in our laboratory have already been published.[25]

Forearm volume was measured according to the water displacement method and drug infusion rates were normalized for 1 dL of tissue by adjusting the concentration of stock solution to the desired infusion rate. Drugs were infused at systemically ineffective rates through separate ports through three-way stopcocks.

Endothelium-dependent vasodilation was evaluated performing a dose–response curve to intra-arterial acetylcholine hydrochloride (Farmigea S.p.A., Pisa, Italy) (cumulative increases of the infusion rates, 0.15, 0.45, 1.5, 4.5, and 15 μg/100 mL forearm tissue/min, for 5 min at each dose).

Endothelium-independent vasodilation was assessed constructing a dose–response curve to intra-arterial infusions of the direct smooth muscle cell relaxant sodium nitroprusside (Malesci, Milan, Italy),[26] freshly dissolved in 5% glucose and protected from light by aluminum foil (cumulative increases, 1.0, 2.0, and 4.0 μg/100 mL forearm tissue/min, for 5 min at each dose). These rates were selected to induce a vasodilation comparable to that obtained with acetylcholine.

Minimal forearm vascular resistance (MFVR), an index of structural changes of the arterial wall,[27] were calculated as the ratio between maximal postischemic forearm blood flow increase (obtained after 13 min of ischemia plus 1 min of hand dynamic exercise) and intra-arterial mean arterial pressure, as described.[28]

The sequence of the experimental interventions was randomized and 45 min of recovery were allowed between the three experimental steps. The operator (AV) was blinded with regard to the results of adhesion molecule assays.

Biohumoral Parameters

In all study subjects the following metabolic parameters were measured on the same day of the study: serum and urinary creatinine, serum triglycerides, total serum cholesterol and HDL cholesterol, and plasma glucose, by conventional clinical chemistry analyses. LDL cholesterol was calculated with the Friedewald's formula.

In all study subjects, a peripheral whole blood sample was obtained in the morning of the study, before the beginning of the infusion experiments, in heparinized syringes through a 19-gauge needle. Platelet-poor plasma was obtained by centrifugation at 3000 rpm for 15 min, then aliquoted and immediately stored at −80°C until the assay. Assays were performed in batch for each adhesion molecule after a single thawing of the aliquoted sample at room temperature and gentle mixing of plasma.

Soluble adhesion molecules E-selectin, ICAM-1, and VCAM-1 were measured by commercially available enzyme-linked immunosorbent assays (Bender, Vienna, Austria, for sE-selectin and sICAM-1, and R&D Systems Europe, Oxon, United Kingdom, for sVCAM-1). Assays were performed in duplicate on duplicate samples, each assayed at least at two different dilutions. The development of a color reaction from the conversion of the chromogenic substrate (tetramethyl-benzidine), directly proportional to the amount of the analyte assayed, was followed for 5 to 20 min with an ELISA plate reader (ETI-System, Sorin Biomedica, Saluggia, Italy) at 450 nm, up to an optimal reading of positive wells. The enzyme reaction was stopped by the addition of 4 N sulfuric acid. Results were calculated by interpolation of a standard curve consisting of at least five measurable points. Intraassay and interassay precision (coefficient of variation) for these assays are $<$5.9% and $<$10.2%, respectively.

Data Analysis

Forearm blood flow data were analyzed as percent increase above baseline level, and comparisons between groups submitted to intra-arterial infusions of acetylcholine or nitroprusside performed by analysis of variance. Differences between groups at the same dose of infusion for each drug were assessed by the Scheffé's test, after demonstration of significant intergroup differences at analysis of variance. Between-group comparisons for minimal forearm vascular resistances and sE-selectin levels were performed by the unpaired Student's *t* test after verification of the parametric pattern of value distributions. The relationships between plasma levels of sE-selectin on the one hand (y variable), and forearm vasodilation responses to acetylcholine and nitroprusside, MFVR, and other biohumoral variables on the other (x variable), were tested by linear simple and multiple regression analysis, and the existence of a significant correlation by the Spearman's correlation coefficient for simple regression and by *t* values for multiple regression analyses, respectively. Analyses were performed with the aid of the Statview statistical

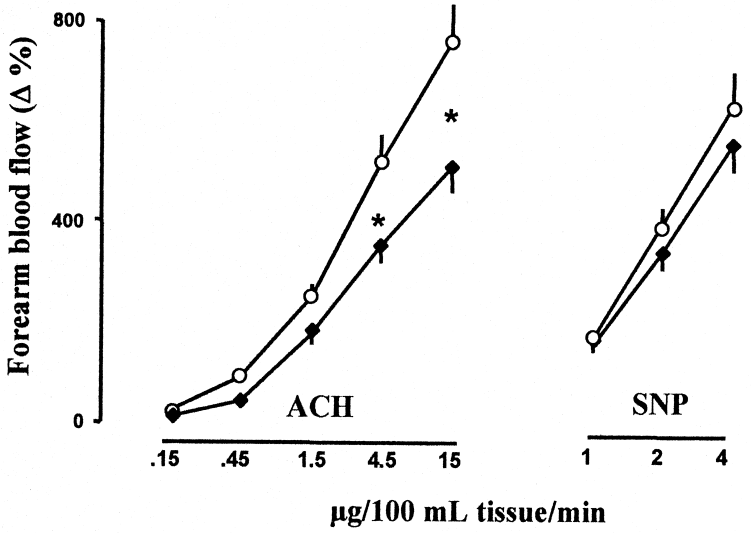

FIG. 1. Dose–response curves of forearm blood flow percentage change from baseline (on the ordinate) to graded intra-arterial infusion of acetylcholine (ACH, **left panel**) and sodium nitroprusside (SNP, **right panel**) in patients with essential hypertension (**closed circles**) and normal controls (**open circles**). Significant differences (*$P < .01$) are found in the response to acetylcholine but not to nitroprusside.

package (Abacus Concepts, Inc., Berkeley, CA). Results were expressed as mean ± SEM.

Results

In hypertensive patients, intra-arterial acetylcholine caused a dose-dependent vasodilation (forearm blood flow increased from to 3.2 ± 0.1 at baseline to a maximum of 19.6 ± 1.5 mL/100 mL forearm tissue/min), which was similar in magnitude to the response to sodium nitroprusside (forearm blood flow increased from 3.2 ± 0.1 to a maximum of 20.1 ± 1.3 mL/100 mL forearm tissue/min). In this study population, the response to the highest acetylcholine infusion rate ranged from 10.9 to 42 μg/100 mL forearm tissue/min. Range of the maximal vasodilating effect of sodium nitroprusside was from 11.4 to 41.7 μg/100 mL forearm tissue/min. Changes in forearm blood flow in response to acetylcholine were smaller in hypertensives as compared to normotensive controls. In this last group forearm blood flow increased from 3.2 ± 0.2 at baseline to a maximum of 26.7 ± 2.5 mL/100 mL forearm tissue/min ($P < .01$ *v* hypertensives). Responses to nitroprusside at doses used was similar in magnitude (from 3.2 ± 0.1 to a maximum of 23.2 ± 1.5 mL/100 mL forearm tissue/min). Differences in nitroprusside responses between hypertensives and normotensives were not significant. Percent increases over baseline after acetylcholine and nitroprusside as a function of the infused doses of each agent are shown in Fig. 1.

Calculated MFVR were 3.0 ± 0.2 U (U = mm Hg/mL × min^{-1}) (range, 1.5 to 4.8 U) in hypertensives, significantly ($P < .001$) higher than in normotensives (1.6 ± 0.1 U; range, 1.2 to 2.0 U). A weak significant correlation ($r = -0.39$, $P < .05$) was found between maximal vasodilation to nitroprusside and MFVR.

Soluble E-selectin plasma levels were significantly higher in hypertensives compared to normotensives (Fig. 2). In hypertensives, significant inverse relations were found between sE-selectin levels and the response to sodium nitroprusside ($r = -0.47$, $P < .01$). No significant correlations were found with the response to acetylcholine ($r = -0.18$) and MFVR ($r = 0.25$) (Fig. 3). Of the demographic, hemodynamic, and metabolic parameters investigated, significant relationships were found between sE-selectin and age ($r = 0.37$, $P < .05$), as well as between sE-selectin and LDL cholesterol ($r = 0.43$, $P < .05$). When all parameters showing significant relations with sE-selectin levels at simple regression analysis were submitted to a multivariate analysis, only responses to nitroprusside and LDL cholesterol remained significant (Table 2), indicating an independent contribution to sE-selectin variability.

Relationship between sE-selectin and results of vascular examinations were also carried out in the pooled population of normotensive and hypertensive controls. Soluble E-selectin remained related to responses to nitroprusside ($r = -0.34$, $P < .05$), LDL cholesterol ($r = 0.40$, $P < .005$), and also, in this case, to MFVR ($r = 0.46$, $P < .001$), mean BP ($r = 0.31$, $P < .05$), and also, this time, to responses to acetylcholine ($r = -0.29$, $P < .05$). The strength of the correlation between sE-selectin and responses to acetylcholine remained in any case weaker than between sE-selectin and correlates of structural vascular changes (responses to nitroprusside or MFVR). An analysis to examine the independent correlates of responses to acetylcholine showed that this was also significantly related only to responses to nitroprusside ($r = 0.5$, $P < .02$). At multivariate analysis in the pooled population, significant determinants of sE-selectin variability remained only for LDL cholesterol ($P = .02$) and MFVR ($P = .05$).

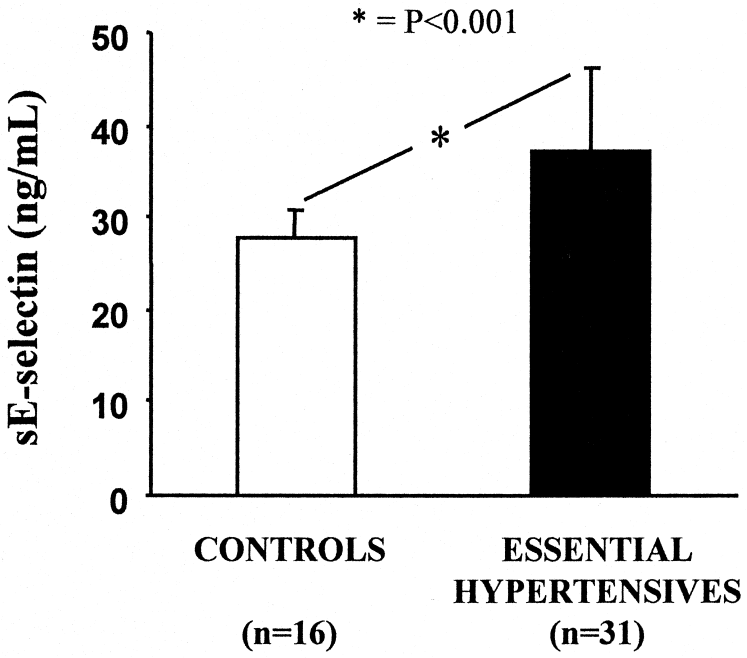

FIG. 2. Plasma concentrations of sE-selectin in normal controls (**left bar**) and in patients with essential hypertension (**right bar**). sE-selectin levels are significantly higher ($P < .001$) in patients compared to controls.

Soluble ICAM-1 and VCAM-1 levels were 395.5 ± 119.0 ng/mL and 586.2 ± 209.3 ng/mL, respectively, not significantly different from healthy controls (370 ± 145 ng/mL and 550 ± 225 ng/mL, respectively). Neither sVCAM-1 nor sICAM-1 were related to any significant extent to maximal forearm blood flow responses to either acetylcholine or nitroprusside, nor were they related to MFVR (data not shown).

Discussion

In the present study we readdressed the issue of levels of E-selectin (and other soluble adhesion molecules ICAM-1 and VCAM-1) in essential hypertension and sought to gain insight as to reasons for their possible increase in this disease. We specifically tested the hypothesis that increases are related to the state of endothelial dysfunction, well characterized in this disease.[29] Indeed, the reduced availability of nitric oxide (NO), possibly because of an increase in oxidative stress,[30] could result in increased expression of endothelial adhesion molecules.[31] Because sE-selectin was the only adhesion molecule showing increased plasma concentrations in hypertensives compared to normotensives, we evaluated the possible relationship between this molecule and the endothelium-dependent response to acetylcholine in the forearm of essential hypertensive patients. The degree of vasodilation induced by acetylcholine is considered an index of endothelial dysfunction.[4,5,7,8] Hypertensive patients, because of the hemodynamic load caused by high BP, may also develop structural vascular changes that reduce the vessel capacity to dilate.[32] These alterations can be detected by evaluating the response to agonists that act directly on smooth muscle cells, such as nitroprusside,[26] or calculating MFVR.[28] These structural vascular modifications can be reversed by antihypertensive treatment.[33] Therefore, to avoid possible chronic therapy-related effects, in the present study we only recruited patients with recent onset of essential hypertension who had never been treated.

In this selected study population we found a significant reduction in acetylcholine-induced vasodilation compared to normotensive controls, indicating the presence of endothelial dysfunction, and a significant increase in MFVR, therefore showing some evidence of structural vascular changes despite the recent onset of disease. We here confirm different values of sE-selectin in hypertensives as compared to normotensives, and "normal" values of other soluble adhesion molecules.[17–21] However, and to some surprise, in hypertensives we found a relatively strong correlation of sE-selectin values with MFVR response to nitroprusside, but not to acetylcholine. Significant correlations of E-selectin levels also with age and LDL cholesterol at simple regression analysis were found. At multiple regression analysis, only responses to nitroprusside and LDL cholesterol remained significant, indicating an independent contribution to sE-selectin variability. These data would indicate that structural vascular changes are more important determinants of sE-selectin levels than impairment of endothelium-dependent vasodilation, as assessed by the acetylcholine testing.

Our search for a relationship of sE-selectin with the vasodilating capacity to acetylcholine was based on the background that an impairment of the vasodilating capacity to acetylcholine would largely reflect impaired NO

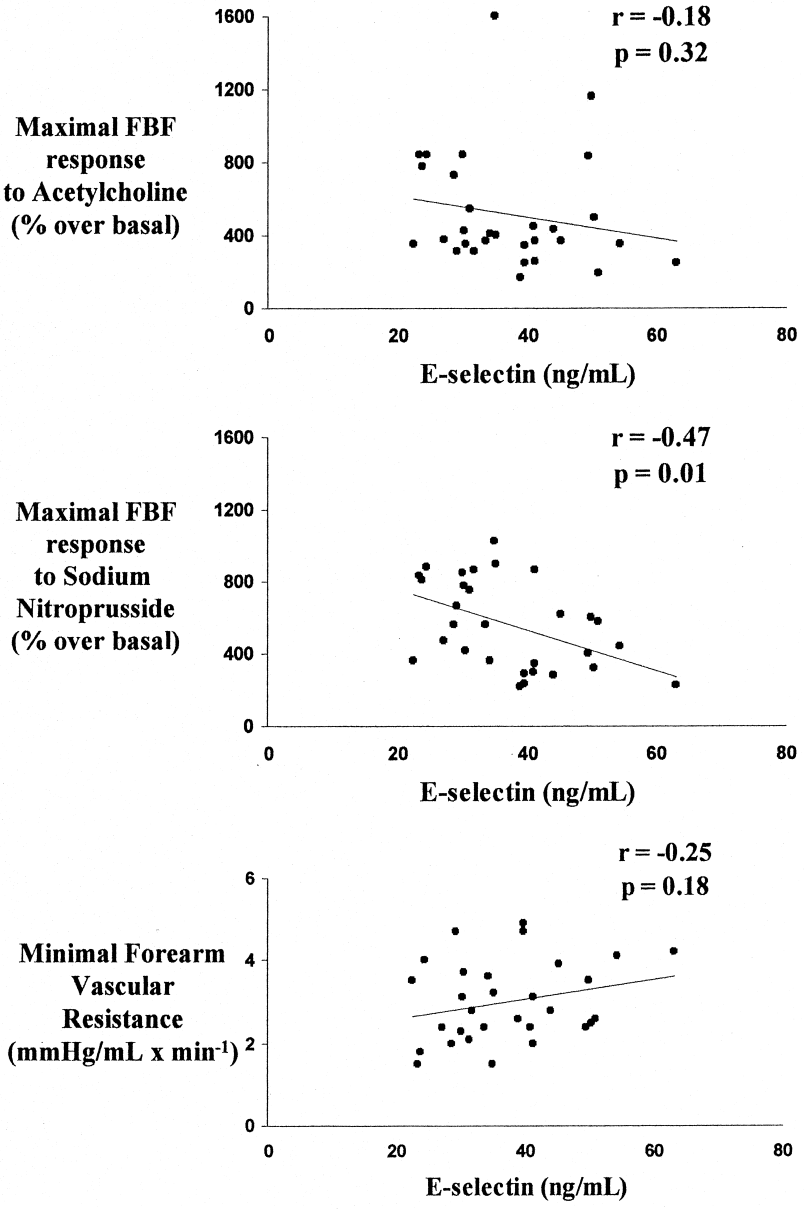

FIG. 3. Linear regression analysis of the maximal forearm blood flow (FBF) responses to acetylcholine (**upper panel**) and to sodium nitroprusside (**middle panel**), and of the minimal forearm vascular resistances (**lower panel**) (all on the y axis), as a function of E-selectin plasma levels (x axis) in patients with essential hypertension. A significant inverse relationship is found between maximal forearm blood flow responses to nitroprusside and E-selectin levels.

availability,[1,4] and that NO, in turn, inhibits endothelial activation, including the expression of E-selectin.[31] Indeed, when the normotensive and the hypertensive subjects are pooled together (a procedure justified by the fact that they were extremely comparable as to most variables tested apart from hypertension and hypertension-related variables), some significant inverse relationship of sE-selectin levels and the maximum vasodilatory capacity to acetylcholine does appear. However, the correlation ($r = -0.30$, $P = .04$) remains weaker than for another index of structural vascular changes, MFVR ($r = 0.47$, $P < .001$), and disappears at multivariate analysis. The existence of some relationship between indices of structural and functional changes at least partially accounts for these significances at univariate analysis. Thus, despite the existence of a theoretical and experimental background relating endothelial dysfunction, as a result of decreased NO availability, to endothelial activation, as evidenced by an increase in circulating E-selectin, we conclude that this relationship is weak or absent within the population studied.

This unexpected finding can be tentatively explained by the following hypotheses. First, vasodilating response to acetylcholine in hypertensive patients is not mediated by NO and, therefore, different degrees of vasodilation induced by acetylcholine cannot identify different degrees of NO availability. This is supported by the finding that acetylcholine vasodilation in hypertension is resistant to NO synthase inhibitors such as N^G-monomethyl-L-argi-

Table 2. Multiple regression analysis of sE-selectin (dependent variable) versus three independent variables: response to nitroprusside (as % increase over baseline), age, and LDL cholesterol (LDL-C) in subjects with essential hypertension (n = 31)

Independent Variable	Regression Coefficient	*t* Value	Probability Level	Decision (5%)
Intercept	20.5	2.13	0.04	
Nitroprusside	−0.019	−3.30	0.03	Reject H_0
Age	0.210	1.18	0.25	Accept H_0
LDL-C	0.142	2.30	0.03	Reject H_0
R^2	0.45			

H_0 denotes the hypothesis that the independent variable does not correlate significantly with E-selectin levels when all independent variables are considered together. Rejecting H_0 indicates that the variable does contribute independently in explaining E-selectin variability.

nine,[34] so that other mediators, among which endothelium-dependent hyperpolarizing factor, are possibly involved.[1] Second, this unexpected lack of relationship might be a district-specific feature of the forearm vascular bed; we cannot exclude that endothelium-dependent vasodilation in other vascular beds, such as the brachial artery or epicardial coronary arteries, could yield different results. Third, a prevailing microcirculatory source of E-selectin, correlated with the greater extension of capillary versus arterial endothelium, could weakly correlate with a prevailing arteriolar response such as the response to acetylcholine.

Our data, on the other hand, show a much more robust relationship of E-selectin plasma levels with indices of structural microvascular changes. Interpretations for this are only tentative and highly speculative at the moment. First, the possibility might exist that the same stimuli able to increase E-selectin expression in vascular endothelium, such as the "inflammatory" cytokines interleukin-1 and tumor necrosis factor, are also able to cause or contribute to microvascular structural alterations, including smooth muscle cell remodeling and collagen synthesis.[35,36] Second, increased E-selectin expression, by fostering leukocyte accumulation, might be a mechanism itself of structural vascular alterations in large arteries, because of the wide array of enzymatic products and secondary cytokines produced by in emigrated leukocytes.[35]

The relatively strong correlation with LDL cholesterol, maintained in the multivariate analysis, could also imply NO-independent effects, such as effects of oxidized/modified LDL on adhesion molecule expression.[37,38] This correlation appears actually stronger than that of sE-selectin with mean arterial pressure, which becomes nonsignificant in the pooled group of normotensive and hypertensive subjects.

Our results contribute to the interpretation of the scattering of values for sE-selectin in essential hypertension. Although this new marker is on the average higher in hypertensive populations, the interest for it in the future will be strictly dependent on the incremental information it will be able to yield in stratifying patients for diagnostic purposes and possibly in having an impact on prognosis. This has been recently demonstrated in coronary artery disease for another soluble adhesion molecule, sICAM-1.[39] Interestingly, to this regard, two recent independent contributions have reported on higher levels of sE-selectin in hypertensive subjects with impaired glucose tolerance,[20,21] where a higher degree of vascular changes are expected to develop, or in patients showing disease progression.[20] The demonstration of a relationship with early structural vascular alterations, as given here, is in line with these reports and with the hypothesis that this marker may give incremental information for diagnostic purposes, for which more understanding is needed with additional investigations.

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