Increased serum laminin and angiogenin concentrations in patients with peripheral arterial occlusive disease

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Abstract

Aim—To measure serum laminin and angiogenin concentrations in patients with peripheral arterial occlusive disease (PAOD) Fontaine stages IIb, III, and IV.

Methods—The study population comprised 38 patients (20 men and 18 women) with stage IV PAOD, 11 patients (six men and five women) with stage III PAOD, 18 patients (10 men and eight women) with stage IIb PAOD, and 23 patients (10 men and 13 women) with deep vein thrombosis. Fifteen normal subjects (matched for risk factors) and 10 patients (five men and five women) without PAOD served as controls. Serum samples were obtained at admission and serum laminin and angiogenin concentrations were measured using an enzyme linked immunosorbent assay.

Results—Patients with stage IV PAOD had higher serum laminin (mean $\pm$ SEM; 826 ± 97 ng/ml) and angiogenin concentrations (467 ± 26 pg/ml) than normal subjects (laminin: 379 ± 21 ng/ml; angiogenin: 358 ± 16 pg/ml) and patients without PAOD (laminin: 277 ± 34 ng/ml; angiogenin: 406 ± 25 pg/ml). A significant correlation was found between angiogenin and laminin and between serum laminin and fibrinogen concentrations in patients with stage IV disease.

Conclusions—Raised laminin and angiogenin concentrations may be indicators of endothelial damage caused by reduced vascular perfusion or compensatory revascularisation, or both.

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damage, systemic sclerosis,⁴ Graves' disease,⁵ and in hepatic schistosomiasis.⁶ Basement membrane degradation is related to serum laminin P1 in patients with transitional cell carcinoma of the bladder.

Angiogenesis, the formation of new blood vessels, occurs by the outgrowth of new capillaries from established blood vessels,⁷ and can be induced by a variety of proteins including basic fibroblast growth factor, laminin and angiogenin.⁸ Angiogenin, a 14.4 kilodalton single chain basic protein, is a potent inducer of neovascularisation; however, its mechanism of action is not known as yet.^{9,10} It has not been shown conclusively to promote endothelial cell proliferation, but it seems to interact with these cells via specific receptors.

Methods

The study population comprised 38 patients (20 men and 18 women) with stage IV PAOD (rest pain, incipient peripheral ulcer and ischaemia of less than one month's duration), 11 patients (six men and five women) with stage III PAOD (rest pain—that is, ischaemic pain, usually occurring at night when blood pressure is critically low), 18 patients (10 men and eight women) with stage IIb PAOD (claudicatio intermittens), and 23 patients (10 men and 13 women) with deep vein thrombosis. Fifteen normal subjects (matched for risk factors, such as hypertension, diabetes mellitus, smoking) and 10 patients (five men and five women) without PAOD served as controls.

All patients with PAOD underwent intra-arterial digital subtraction angiography or colour coded duplex ultrasonography (the combination of real time and Doppler ultrasonography) to determine the location and degree of stenoses. Patients with deep vein thrombosis underwent phlebography or colour coded duplex ultrasonography to determine the location and extent of venous thrombosis.

Routine laboratory investigations included red blood cell count, haematocrit, white blood cell count, platelet count, serum electrolytes, total bilirubin, liver enzymes, serum creatinine, cholesterol, triglycerides, fibrinogen, and C-reactive protein. Serum samples for determination of soluble laminin and angiogenin were obtained from each patient at admission.

Serum concentrations of laminin and angiogenin were measured using commercially available enzyme linked immunosorbent assays (ELISA) (Takara Shuzo, Kyoto, Japan, and

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In patients with peripheral arterial occlusive disease (PAOD) endothelial damage is frequently caused by hypertension, diabetes mellitus or other metabolic disorders. The capillary basement membrane is characteristically thickened and subtotal or total vessel occlusion leads to revascularisation and development of collateral blood vessels.

Laminin is a ubiquitous basement membrane component which plays a central role in maintaining the structure and function of basement membranes.^{1,2} Serum laminin concentrations are raised in patients with hepatic fibrosis, various tumour types,³ glomerular

Quantikine, R and D systems, Minneapolis, USA, respectively) as recommended by the manufacturers.

STATISTICAL ANALYSIS

The following statistical tests were used: analysis of variance, the Tukey test and Pearson's correlation matrix. All analyses were two tailed, and $p < 0.05$ was considered significant.

Results

The mean (SD) age and weight, respectively, of patients with stage IV PAOD were 59 (14) years and 68 (19) kg. The mean arterial ankle pressure at the posterior tibial artery was 39 (33) mmHg and the mean Doppler index was 0.30 (0.24). Twenty two of the 38 patients had diabetes mellitus, 16 smoked and 18 had hypertension. The mean age and weight, respectively, of patients with stage III PAOD were 61 (13) years and 70 (13) kg. Six patients had diabetes mellitus, eight smoked and eight had hypertension. Patients with stage IIb PAOD had a mean age of 58 (14) years and mean weight of 72 (10) kg. Twelve patients had diabetes mellitus, 10 smoked and 10 had hypertension. Patients with deep vein thrombosis had a mean age of 45 (15) years and a mean weight of 60 (12) kg. Eight patients had diabetes mellitus, 10 had hypertension and 10 smoked. Three patients had deep vein thrombosis of the arm, and the others of the leg.

The mean age of the normal controls (volunteers) was 55 (10) years with a mean weight of 65 (14) kg. Seven had diabetes mellitus, eight had hypertension and 10 smoked. In the patient control group the underlying diseases were pneumonia, cardiomyopathy, chronic obstructive pulmonary disease, and fever of unknown origin. Their mean age was 58 (9) years, their mean weight was 70 (11) kg and 10 smoked.

Patients with stage IV PAOD had significantly higher serum angiogenin (mean $\pm$ SEM: 467 ± 26 pg/ml) and laminin concentrations (826 ± 97 ng/ml) than either the volunteer (angiogenin, 358 ± 16 pg/ml; laminin, 379 ± 21 ng/ml) or the patient control groups (angiogenin, 406 ± 25 pg/ml; laminin, 277 ± 34 ng/ml) (table 1). In patients with stage III PAOD the mean serum laminin and angiogenin concentrations were 644 ± 153 ng/ml and 375 ± 26 pg/ml, respectively. In patients with stage IIb PAOD the corresponding values were 680 ± 97 ng/ml and 374 ± 15.4 pg/ml, respectively, and in those with deep vein thrombosis these values were 534 ± 61 ng/ml and 355 ± 24 pg/ml, respectively.

In patients with stage IV PAOD there was a significant correlation between angiogenin and laminin concentrations ($r = 0.4667$; $p < 0.016$) and between laminin and serum fibrinogen concentrations (456 ± 172 mg/dl) ($r = 0.6294$; $p < 0.028$).

Discussion

Atherosclerosis is characterised by a focal intimal thickening of medium and large sized arteries. More than 250 factors have been implicated in the pathogenesis of this condi-

Table 1 Mean $\pm$ SEM serum laminin (ng/ml) and angiogenin (pg/ml) concentrations in patients with PAOD and deep vein thrombosis (DVT)

Subject group	Laminin	Angiogenin
PAOD		
stage IV	$826 \pm 97^*$	$467 \pm 26^*$
stage III	644 ± 153	375 ± 26
stage IIb	680 ± 97	374 ± 15
DVT	534 ± 61	355 ± 24
Controls		
patients	277 ± 34	406 ± 25
volunteers	379 ± 21	358 ± 16

* $p < 0.05$ v both control groups.

tion,^{11 12} and there is little doubt that fibrinogen is a major independent cardiovascular risk factor.^{13 14} Population studies have demonstrated an association between fibrinogen concentrations and severity of disease and the numerous pathophysiological mechanisms by which fibrinogen contributes to atherothrombogenesis.^{15 16} However, there is no evidence that an increase in the fibrinogen concentration is directly responsible for the vascular disease, as the cytokines which participate in the synthesis of fibrinogen by hepatocytes, such as interleukin-6, could also induce endothelial cell damage by increasing the production of tumour necrosis factor. In the present study raised plasma fibrinogen concentrations were detected in patients with stage IV PAOD, as has been found by other authors. These patients also had significantly raised serum laminin concentrations compared with values measured in both control groups, strongly suggesting that these increased values were not an epiphenomenon. Although patients with stage III and IIb PAOD, and those with deep vein thrombosis had numerically higher serum laminin concentrations compared with the control groups, the difference was not significant. A significant correlation was found between plasma fibrinogen and serum laminin concentrations in patients with stage IV PAOD.

Laminin is a ubiquitous component of basement membranes and plays an important role in maintaining their structure and function.^{2 17} Laminin is relatively resistant to degradation by proteases, and it is possible that it is formed and released into the circulation during normal basement membrane degradation. Increased serum concentrations measured in patients with PAOD, however, might reflect active synthesis of basement membrane proteins throughout the body. Laminin might therefore be an indicator of endothelial cell damage,^{4 18} or compensatory tissue repair induced by several mechanisms involved in the pathogenesis of PAOD, or both, and therefore could be used as a marker for severity of disease.

Subtotal or total vessel occlusion, induced by thickening of the vessels, leads to revascularisation and the development of collaterals. Angiogenesis, the formation of new blood vessels, occurs by the outgrowth of new capillaries from established blood vessels through a process that involves digestion of the extracellular matrix and subsequent migration of the endothelial cells into tube-like structures. Angiogenesis can be induced by a variety of

proteins including angiogenin.⁸ In the present study we found significantly increased serum concentrations of angiogenin in patients with stage IV PAOD compared with both control groups, patients with stage IIb PAOD and those with deep vein thrombosis. Increased production of angiogenin may be indicative of compensatory angiogenesis in these patients. Revascularisation and the development of collaterals are important mechanisms of compensation in patients with PAOD.

In summary, serum laminin and angiogenin concentrations are increased in patients with stage IV PAOD and may be used as markers of disease severity. Subtotal or total vessel occlusion, endothelial damage, tissue repair, and adaptive revascularisation may contribute to increased serum concentrations of both markers.

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