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Short communication

Correlation between increasing tissue ischemia and circulating levels of angiogenic growth factors in peripheral artery disease

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ABSTRACT

Introduction: The aim of the present study was to assess the circulating levels of vascular endothelial growth factor (VEGF) and other suggested therapeutic growth factors with the degree of ischemia in patients with different clinical manifestations of peripheral arterial disease (PAD) according to the Rutherford grades.
Methods: The study cohort consists of 226 consecutive patients admitted to a Department of Vascular Surgery for elective invasive procedures. PAD patients were grouped according to the Rutherford grades after a clinical assessment. Ankle-brachial pressure indices (ABI) and absolute toe pressure (TP) values were measured. Serum levels of circulating VEGF, hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), and platelet derived growth factor (PDGF) were measured from serum and analysed against Rutherford grades and peripheral hemodynamic measurements.
Results: The levels of VEGF ($P = 0.009$) and HGF ($P < 0.001$) increased significantly as the ischaemic burden became more severe according to the Rutherford grades. PDGF behaved in opposite manner and declined along increasing Rutherford grades ($P = 0.004$). A significant, inverse correlations between Rutherford grades was detected as follows; VEGF (Pearson's correlation = 0.183, $P = 0.004$), HGF (Pearson's correlation = 0.253, $P < 0.001$), bFGF (Pearson's correlation = 0.169, $P = 0.008$) and PDGF (Pearson's correlation = 0.296, $P < 0.001$). In addition, VEGF had a clear direct negative correlation with ABI (Pearson's correlation −0.19, $P = 0.009$) and TP (Pearson's correlation −0.20, $P = 0.005$) measurements.
Conclusions: Our present observations show that the circulating levels of VEGF and other suggested therapeutic growth factors are significantly increased along with increasing ischemia. These findings present a new perspective to anticipated positive effects of gene therapies utilizing VEGF, HGF, and bFGF, because the levels of these growth factors are endogenously high in end-stage PAD.

1. Introduction

An ischemic non-healing wound in the leg characterises threatening limb loss. Without intensive revascularisation procedures and extensive wound care, limb loss is often imminent. Both endovascular and open revascularisation and secondary prevention measures have been extensively studied [1], but the knowledge of the molecular mechanisms associated with end-stage PAD still remain limited.

The role of several vascular endothelial growth factor (VEGF)-associated growth factors in angiogenesis have been widely studied in various pathological and experimental conditions, and have been shown to be possible targets of medical angiogenic therapy [2,3]. Hypoxia has been shown to induce angiogenesis in malignant tumours via the expression of the several angiogenic growth factors including VEGF and basic fibroblast growth factor (bFGF) [2–4]. In addition to the central role of VEGF-related growth factors in malignant and pathological states, these growth factors play a critical role in the physiological response of various tissues to hypoxia [3]. Although new gene based therapies promoting growth factors, such as VEGF, are applied to ischemic tissues appear promising [5], the levels of VEGF-related growth factors have not been investigated in the various clinical presentations of peripheral artery disease (PAD). We assessed the possible role of VEGF and other suggested therapeutic angiogenic growth factors in patients presenting with different degrees of limb ischemia.

2. Methods

2.1. Study cohort

The present study is part of our ongoing the PURE ASO Study [6].

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The patient cohort comprised of consecutive patients that were admitted to the Vascular Department in the Turku University Hospital for either endovascular or operative treatment of PAD. During enrolment period 227 suitable patients were screened. One patient declined and therefore 226 patients gave their written informed consent. Ankle-brachial pressure indices (ABI) and absolute toe pressure values (TP) were measured. Limb ischemia was scored in all patients according to the Rutherford grades upon admission to the study and the patients were sorted into groups accordingly. Pre-treatment serum samples were collected from each patient. A complete medical history including the patient's cardiovascular risk factors was collected from the medical records and intense interviews.

As a reference group, serum was collected from 20 middle-aged healthy adults. A comprehensive medical history was obtained and a duplex ultrasound of the lower limb arteries was performed to ensure that there were no detectable atherosclerotic lesions.

This study was approved by local ethical committee of south-western Hospital district of Finland.

2.2. Sample processing and analysis of cytokines, chemokines, and growth factors

All blood samples were collected from cubital vein after fasting for at least 6 hr. Whole blood samples (9 mL) were collected in serum tubes. The collected samples were left to clot at room temperature during transport to the MediCity Research Laboratory, University of Turku. Samples were centrifuged at 2000*g* for 10 min at room temperature on arrival followed by the removal of serum. Serum samples were stored at −70 °C. Analysis was performed simultaneously using an identical magnetic bead suspension array kit from the Bio-Plex Pro Human Cytokine 21- and 27-plex panels (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions, with the exception of the use of half of the recommended amount of beads, and concentration of detection antibodies and streptavidin–phycoerythrin conjugate as presented previously [6]. Results were analysed using the Bio-Plex 200 System and calculated using the Bio-Plex Manager 6.0 software (Bio-Rad Laboratories).

2.3. Statistical analyses

For statistical analyses, the patients were grouped according to the Rutherford grades. The reference group with no evident PAD was used as a baseline value. Statistical analyses were performed with IBM (NYC, USA) SPSS version 22. To test for significant differences between Rutherford grades, the one-way analysis of linearity (ANOVA) test was used. Normal distribution of the data was tested using the Shapiro-Wilk test. If necessary, the data were log-transformed to normal distribution and then re-tested using the same one-way ANOVA. The linear association of all growth factors were also directly assessed against ABI and TP measurements using Pearson's correlation for log-transformed normally distributed data. A P value < 0.05 was considered as statistically significant.

3. Results

Patient characteristics according to the Rutherford grades are depicted in Table 1. Compared to the Rutherford grade 0 all other demographic variables but gender, systolic pressure and creatinine value were significantly different between Rutherford grades.

Angiogenic growth factor measurements were skewed and tailing to very high values. Thus, throughout the statistical analyses log-transformed values of growth factors were used. VEGF had a significant positive linear association with increasing ischemia according to Rutherford grades (R = 0.183, $P = 0.004$) (Fig. 1A). With increasing ischemia a positive linear association of HGF levels was also detected (R = 0.253, P < 0.001) (Fig. 1B), but either toe pressure or ABI indices were did not show significant association with HGF levels (data not shown). Although a significant correlation between bFGF levels and Rutherford grades (R = 0.169, P = 0.004; Fig. 1C), ABI (R = −0.15, P = 0.039; Fig. 1G) and toe pressure (R = −0.17, P = 0.016; Fig. 1H) was detected, the Pearson's correlation value did not reach required ≥ 0.170 for the size of the study cohort. The PDGF levels had a declining linear association with and Rutherford grades (Pearson's correlation = −0.296, P < 0.001) (Fig. 1D). However, PDGF did not significantly correlate with ABI or TP(Data not shown). In the context of this patient population age is the strongest driver of PDGF[6]. PDGF has a significant negative correlation with increasing age (Pearson's correlation −0.25, $P = 0.002$) (Data not shown).

Table 1
Patient characteristics of the study groups according to the Rutherford grades.

Rutherford grade	0	1	2	3	*P* value*
N	27	100	57	46	NS
Male (%)	48%	56%	59%	59%	NS
Age (years)	45 (SD, 17)	68 (SD, 9.5)	72 (SD, 11)	75 (SD, 12)	< 0.01
History of smoking	7%	72%	65%	40%	< 0.01
Hypertension	7%	73%	72%	70%	< 0.01
Dyslipidemia	7%	41%	33%	20%	< 0.01
Diabetes	4%	26%	37%	47%	< 0.01
CKD	0%	11%	20%	45%	< 0.01
ABI	NA	0.52 (SD, 0.19)	0.43 (SD, 0.26)	0.38 (SD, 0.32)	< 0.01
TP (mmHg)	NA	51 (SD, 24)	31 (SD, 17)	28 (SD, 23)	< 0.01
Systolic BP (mmHg)	121 (SD, 8)	141 (SD, 35)	153 (SD, 48)	156 (SD, 39)	NS
Total cholesterol (mmol/L)	5 (SD, 1.2)	4.6 (SD, 1.3)	4.5 (SD, 1.2)	3.8 (SD, 0.77)	< 0.01
HDL	2.9 (SD, 0.95)	2.4 (SD, 0.97)	2.4 (SD, 1.1)	1.9 (SD, 0.69)	< 0.01
LDL	1.7 (SD, 0.55)	1.5 (SD, 0.52)	1.5 (SD, 0.62)	1.3 (SD, 0.42)	0.011
Trigly	2.4 (SD, 1.3)	1.5 (SD, 1.0)	1.3 (SD, 0.60)	1.3 (SD, 0.58)	< 0.01
Creatinine (μmol/L)	80 (SD, 18)	83 (SD, 27)	100 (SD, 130)	110 (SD, 47)	NS

CKD, chronic kidney disease; ABI, ankle-brachial index; TP, systolic toe pressure; PB, blood pressure.

* Continuous variables Oneway ANOVA; Categorical variables Fisher's exact test.

4. Discussion

Although VEGF-associated growth factors have been intensively examined in various pathophysiological conditions and animal models [7] and they have shown potential to promote angiogenesis in ischemic tissue, they have not proven beneficial in the treatment of CLI [5]. Unfortunately, the physiological function of these growth factors in PAD, and particularly CLI, has not been thoroughly characterised. In the present study, serum levels of essential angiogenic growth factors, that have been considered to have therapeutic potential in CLI, were significantly altered with the degree of expressed ischemia. Similar findings have been reported earlier from both blood samples and ischemic lower limb tissue concerning VEGF [8].

In an experimental rat model, bFGF has been shown to reduce heart muscle defects following acute myocardial infarction and to induce elevated expression of VEGF [9]. Both VEGF- and bFGF-induced angiogenesis in a mouse model, and are synergistically activated via the