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Vascular Endothelial Growth Factor Following Myocardial Infarction*

Naczyniowy śródbłonkowy czynnik wzrostu w zawale mięśnia sercowego

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Abstract

Background. VEGF-A plays an important role in the repair processes following acute myocardial infarction (AMI), mainly by the formation of coronary collateral circulation.
Objectives. In present study the concentrations of VEGF-A in plasma ("free" VEGF) and serum ("free" + platelet-derived VEGF) in patients with ST-segment elevation myocardial infarction (STEMI) and in patients with stable angina (SA) were investigated.
Material and Methods. Forty patients with STEMI, 30 patients with stable angina, and 20 healthy volunteers were investigated. Blood from the STEMI patients was sampled at the time of admission, on days 1 and 2, and on discharge. VEGF-A was assayed with an ELISA kit.
Results. The concentrations of VEGF-A in the plasma and serum in the healthy volunteers were 7.9 ± 15.1 pg/ml and 161 ± 123 pg/ml, respectively. The patients with STEMI could be divided in two subgroups based on their VEGF level. In the first subgroup (23 patients) the plasma (25.0 ± 18.4 pg/ml) and serum (143 ± 127 pg/ml) VEGF-A concentrations were not significantly different from those in the healthy volunteers. In the second subgroup (17 patients) the respective concentrations were 84.9 ± 55.5 pg/ml and 608 ± 138 pg/ml, significantly higher ($p < 0.05$) than in the first subgroup. Patients with stable angina exhibited a significantly higher concentration of VEGF-A in serum (358 ± 200 pg/ml), but not in plasma (29.9 ± 30.0 pg/ml), compared with the control group.
Conclusions. Of the 40 patients with STEMI, only 17 exhibited increased VEGF concentration in both serum and plasma, whereas all the patients with SA had increased levels of VEGF in serum, but not in plasma. Ischemic and/or damaged myocardium is not a source of increased VEGF-A concentration in the circulation. Its most likely source in the bloodstream are platelets, which seem to have a role in the physiological storage and/or transport of particles for this cytokine (**Adv Clin Exp Med 2009, 18, 2, 147–152**).

Key words: VEGF-A, myocardial infarction, stable angina, platelets.

Streszczenie

Wprowadzenie. Naczyniowy, śródłonkowy czynnik wzrostu (VEGF-A) pełni istotną rolę w przebiegu procesów wytwarzania krążenia obocznego u pacjentów po zawale mięśnia sercowego.
Cel pracy. Zbadanie stężenia VEGF-A w osoczu (frakcja „wolna") i w surowicy (frakcja płytkowa + frakcja „wolna" VEGF) u pacjentów ze świeżym zawałem mięśnia sercowego i u pacjentów ze stabilną chorobą wieńcową.
Materiał i metody. Badaniami objęto 40 pacjentów z zawałem mięśnia sercowego z uniesieniem odcinka ST, 30 pacjentów ze stabilną chorobą wieńcową oraz 20 zdrowych ochotników. Krew do badań pobierano na skrzep oraz na EDTA w momencie przyjęcia do szpitala oraz u pacjentów z zawałem po upływie 24, 48 godz., a także tuż przed wypisem. Stężenie VEGF oznaczano techniką ELISA.
Wyniki. Stężenie VEGF w osoczu i surowicy zdrowych ochotników wynosiło odpowiednio: 7,9 ± 15,1 pg/ml i 161 ± 123 pg/ml. Wśród pacjentów z zawałem można wydzielić dwie podgrupy. W pierwszej podgrupie (23 pacjentów) stężenie VEGF-A w osoczu (25,0 ± 18,4 pg/ml) i surowicy (143 ± 127 pg/ml) było podobne jak w grupie kontrolnej. W podgrupie drugiej (17 pacjentów) natomiast stężenia VEGF-A wynosiły: w osoczu 84,9 ± 55,5 pg/ml, a w surowicy 608 ± 138 pg/ml. Wartości te były statystycznie znamiennie większe ($p < 0,05$) w porównaniu z wartościami u pacjentów z pierwszej podgrupy. Pacjenci ze stabilną chorobą wieńcową wykazywali znacząco większe

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stężenie VEGF-A w surowicy (358 ± 200 pg/ml), ale nie w osoczu (29,9 ± 30 pg/ml) w porównaniu do stężenia VEGF-A w grupie kontrolnej.
Wnioski. Spośród 40 pacjentów z zawałem mięśnia serca, jedynie u 17 stwierdzono znamiennie wyższe stężenia VEGF zarówno w surowicy, jak i osoczu, podczas gdy u pacjentów ze stabilną chorobą wieńcową obserwowano większe stężenia VEGF jedynie w surowicy. Uzyskane wyniki wskazują na zróżnicowaną odpowiedź pacjentów z zawałem na wywołaną niedokrwieniem syntezę VEGF. Źródłem VEGF-A w krążeniu są prawdopodobnie głównie płytki krwi, a nie uszkodzone niedokrwieniem miokardium (**Adv Clin Exp Med 2009, 18, 2, 147–152**).

Słowa kluczowe: VEGF-A, stabilna choroba wieńcowa, zawał serca, płytki krwi.

During the repair processes following acute myocardial infarction, the formation of coronary collateral circulation is often observed. The development of coronary collaterals is an important adaptive process following myocardial ischemia. Increased collateral perfusion can limit myocardial ischemia, prevent myocardial necrosis, and attenuate myocardial dysfunction [1]. The mechanisms leading to collateral development are incompletely understood. However, enhancement of the collateral circulation involves both enlargement and remodeling of preexisting collaterals, i.e. arteriogenesis and the formation of new capillaries, i.e. angiogenesis [2].

Vascular endothelial growth factor (VEGF-A), the most potent angiogenic cytokine known, is associated with many physiological and pathological neovascular events. Four VEGF isoforms are generated from a single gene by alternative splicing [3, 4]. VEGF-A, a 46-kDa dimeric peptide, can stimulate angiogenesis, endothelial regeneration, and vascular permeability *in vivo* [5]. Most importantly, gene expression of VEGF-A is strongly increased under hypoxic conditions *in vitro* and in the ischemic myocardium *in vivo*. Elevated VEGF-A levels have also been found in patients with atherosclerotic risk factors, including hypertension and diabetes [3, 6]. Many stimuli, including growth factors, hormones, cytokines, and cellular stress, regulate VEGF-A expression, particularly at the transcriptional level [7]. It has also been found that hydrogen peroxide markedly stimulates the autocrine expression of VEGF-A mRNA and VEGF-A protein [8]. This study reveals a protective mechanism of endothelial cells against injury involving autocrine VEGF-A production and its cytoprotective role. Recently published studies demonstrate that ischemia/reperfusion injury induces VEGF-A and its receptors in the porcine heart and has a potential role in the cardiac remodeling process [9]. On the other hand, some studies suggest that the origin of the elevated serum VEGF-A is most likely platelets rather than the infarcted myocardium [10, 11]. Therefore the present study was designed to determine the plasma and serum VEGF-A levels at different time points after AMI onset and in patients with stable angina. It was believed that the plasma concentration of VEGF-A may reflect its release from the infarcted myocardium and eventually from activated platelets. The serum level, in contrast, also reflects the VEGF-A released from platelets during *in vitro* clotting.

Material and Methods

Forty consecutive patients (28 males and 12 females, average age: 62.3 years) presenting with acute myocardial infarction with ST segment elevation (STEMI) were included in the study. All the patients underwent primary coronary intervention for reperfusion. Clopidogrel loading (300 mg) and aspirin (300 mg) were administered at the time of coronary intervention. During the procedure, unfractioned heparin (100 IU/kg) was administered according to standard practice. Exclusion criteria for the patients were malignant diseases, inflammatory diseases, diabetes mellitus, and severe kidney failure. Blood samples were drawn immediately after hospital admission (before any interventions) and, in patients with STEMI, also on days 2 and 3 and the day of discharge (usually 8 days after AMI). The data were compared with age-matched control subjects (n = 20) and with patients with stable angina (n = 30) who were qualified for non-urgent PTCA. The patients with stable angina were under standard therapy, including β-blockers, ACE inhibitors, and statins. The study was approved by the local ethics committee. Formal consent was obtained from the subjects before blood samples were collected. The investigation conformed with the principles outlined in the Declaration of Helsinki.

Blood Sampling

Blood samples were drawn from an antecubital vein. EDTA plasma was obtained after 20-minute centrifugation at 1500 × *g* and 4°C. Parallel blood samples were allowed to clot by incubating whole blood at room temperature for 2 hrs. The samples were centrifuged after 30, 60, and 120 min of incubation. The incubation of the blood

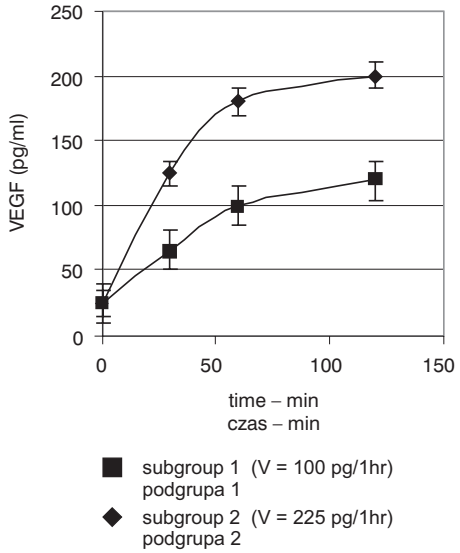

Fig. 1. VEGF release from platelets during clotting *in vitro*
– ■ – Subgroup 1 – patients with STEMI and low VEGF levels in both plasma and serum (n = 23),
– ◆ – Subgroup 2 – patients with STEMI and significantly higher VEGF levels in both plasma and serum (n = 17). Data are expressed as the mean value ± standard deviation

Ryc. 1. Uwalnianie VEGF z płytek krwi podczas wykrzepiania *in vitro*
– ■ – podgrupa 1 – pacjenci ze STEMI i maym zwężeniem VEGF w osoczu i surowicy (n = 23),
– ◆ – podgrupa 2 – pacjenci ze STEMI i większym zwężeniem VEGF w osoczu i surowicy (n = 23).
Wyniki wyrażono jako średnie ± odchylenie standardowe

samples led to elevation of the VEGF-A concentration, most likely reflecting the release of VEGF-A from platelets *in vitro* (Fig. 1). During incubation, no sign of hemolysis was seen. For further evaluation, VEGF-A concentration was determined in serum obtained after 2 hrs of standard incubation, since the VEGF-A concentration reaches a plateau after that clotting time. The plasma and serum samples were stored at –20°C until further analysis.

Laboratory Analysis

VEGF-A concentration was determined by an enzyme linked immunosorbent assay (ELISA). The kits were obtained from R&D Systems (Minneapolis, MN, USA). The sensitivity of the assay was 9 pg/ml. The antibody used in the assay did not exhibit cross-reactivity with either VEGF-A-related or -unrelated peptides. The concentrations of troponin I (cTnI), CK-MB, and hs-CRP were determined using the Dimension reagents and biochemical analyzer from Dade-Behring (Marburg, Germany). A complete blood count using a Sysmex K-1000 automatic analyzer was done to determine platelet count in the plasma samples and in the same blood samples in which VEGF-A was assayed.

Statistical Analysis

Comparison between groups was done using Student's *t* test. Data are expressed as the mean ± standard deviation (*SD*). Changes were considered statistically significant with $p < 0.05$.

Results and Discussion

The mean plasma VEGF-A level in the healthy subjects was 7.9 ± 15.1 pg/ml and in the corresponding serum samples 161 ± 123 pg/ml. Therefore it seems that more than 90% of the VEGF-A molecules in the sera originate from platelets. The same proportion was observed in the patients with stable angina and STEMI. In the patients with stable angina, plasma VEGF-

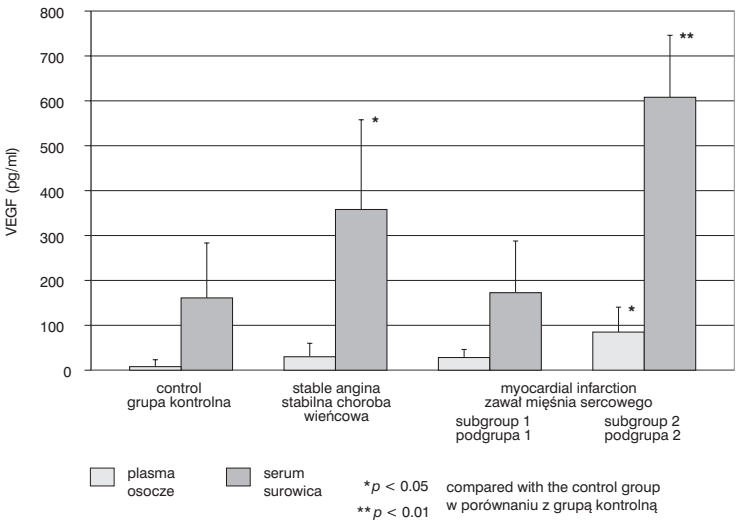

Fig. 2. Plasma and serum VEGF levels in different clinical conditions
Controls: n = 20, stable angina: n = 30. Other details as Fig. 1.

Ryc. 2. Stężenie VEGF w osoczu i surowicy krwi w różnych stanach klinicznych
Grupa kontrolna: n = 20, stabilna choroba wieńcowa: n = 30. pozostałe szczegóły wg ryc. 1.

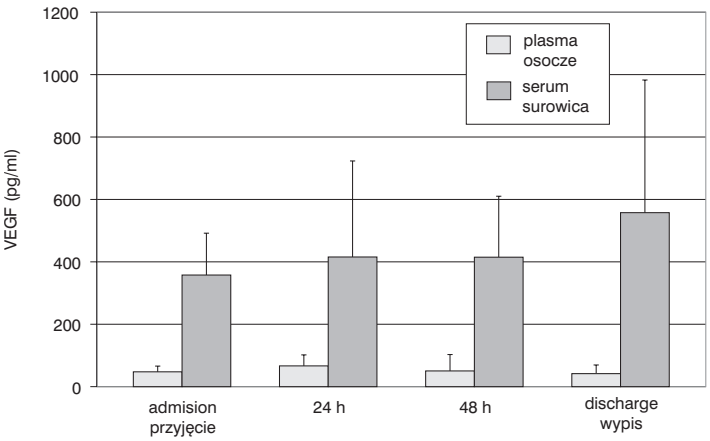

Fig. 3. Plasma and serum VEGF levels after myocardial infarction

Ryc. 3. Stężenie VEGF w osoczu i surowicy krwi po zawale mięśnia sercowego

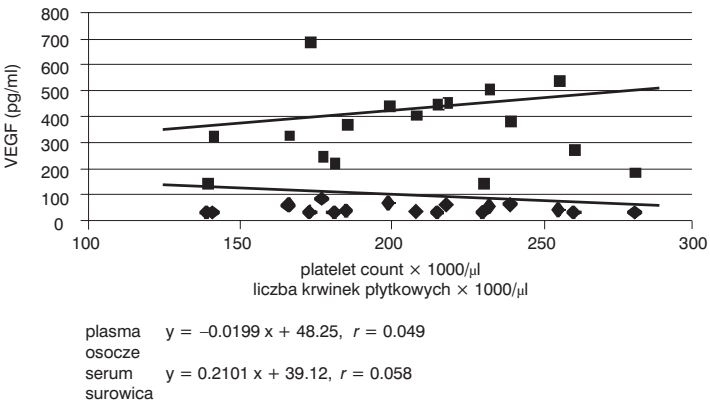

Fig. 4. Relationship between platelet count and VEGF level in plasma and serum

Ryc. 4. Korelacja między liczbą krwinek płytkowych a stężeniem VEGF w osoczu i surowicy krwi

Table 1. Biochemical characteristics of patients with myocardial infarction

Tabela 1. Biochemiczna charakterystyka pacjentów z zawałem mięśnia sercowego

Parameter (Wskaźnik)	Subgroup 1 (Podgrupa 1) n = 23	Subgroup 2 (Podgrupa 2) n = 17	*p*
CK-MB mass	8.7 ± 13.2	11.2 ± 15.5	ns
cTnI	0.44 ± 0.62	0.49 ± 0.76	ns
hs-CRP	0.50 ± 0.75	1.50 ± 2.28	< 0.01

ns – no significance. See text of Fig. 1 for the definition of the patient subgroups.

ns – brak istotności statystycznej. Definicje podgrup pacjentów wg ryc. 1.

A remained at a low level, but the serum concentration was significantly elevated (Fig. 2). It should be emphasized that no platelet activation occurred in these specific clinical conditions. Ferroni et al. [6] recently showed that *in vivo* platelet activation is responsible for the elevated VEGF levels in the plasma of hypertensive patients. Nadar et al. [12], however, did not find any correlation between changes in platelet activation and changes in angiogenic factors.

The patients with STEMI could be divided in two subgroups based on their VEGF-A level. In the first subgroup the plasma and serum VEGF-A concentrations were not significantly different from those of the healthy individuals. In the second subgroup, however, the plasma and serum VEGF-A levels were significantly higher than in the controls. VEGF-A concentrations in the serum samples obtained 24 and 48 hrs after the onset of STEMI remained elevated, even at the time of discharge (Fig. 3). These findings provide evidence that VEGF-A is likely to be an endogenous activator of coronary collateral formation in the human heart following myocardial infarction at least in some patients. In contrast to other investigators [10], the elevated serum and plasma VEGF-A level after myocardial infarction in the present study did not correlate with the number of circulating platelets (Fig. 4).

The kinetics of VEGF-A release during clotting was different in the two subgroups of patients with myocardial infarction (Fig. 1). The velocities of VEGF-A release from platelets were about 100 and 225 pg/hr in the patients of the first and second subgroup, respectively. The maximal concentration of VEGF was seen after 120 min of clotting. The biochemical characterization of the subgroups

of patients with STEMI is summarized in Table 1. The concentrations of biomarkers of myocardial injury, cTnI and CK-MB, were about the same in both subgroups. It may be speculated that the infarcted area is not a factor determining VEGF-A expression. Surprisingly, in the subgroup of patients exhibiting elevated VEGF-A concentrations in both plasma and serum, a higher level of the inflammatory marker hs-CRP was observed. It seems that not only ischemic conditions, but also inflammation may lead to increased production of VEGF-A. Maruyama et al. [13] demonstrated that the pro-inflammatory cytokine interleukin-1β increases VEGF-A production in rat neonatal cardiac myocytes via activation of tyrosine kinases and is involved in the formation of collateral microvessels. On the other hand, VEGF-A produced by different cell types (i.e. endothelial cells) under atherogenic stimulus could be scavenged and stored within platelets and is only later released at the site of thrombosis in result of platelet activation. Several studies suggest a role for platelets as inflammatory cells [14, 15]. Platelets not only release numerous inflammatory mediators, such as chemokines, but may, upon activation, also induce the expression of such substances in other cells (e.g. monocytes/macrophages and granulocytes). Therefore, platelets may be considered the main member of the self-perpetuating pathogenic loop in thrombosis and inflammation.

It is interesting to note that it was possible to demonstrate numerous cases of patients with STEMI in whom the VEGF-A concentrations in plasma and serum samples were the same as in the healthy individuals. In these patients, a lower mean serum concentration of hs-CRP was observed than in the patients who exhibited elevated levels of VEGF-A. These findings are in line with the suggestion that inflammatory mediators are co-responsible for VEGF-A expression in the cardiovascular system. Today, almost all patients with coronary artery disease are administered a daily doses of aspirin. Gerrah et al. [16] demonstrated that aspirin decreases VEGF-A release during myocardial ischemia. VEGF-A level is also significantly lowered by the aspirin treatment of hypertensive patients [10]. Therefore a possible mechanism leading to decreased VEGF-A level in some patients after STEMI could be related to the anti-inflammatory effects of aspirin. This possibility is supported by the results that demonstrated a low level of VEGF-A not only in plasma, but also in serum. The serum concentration of VEGF-A reflects total cytokine level released into the circulation *in vivo* following myocardial infarction and the mainly platelet-stored pool which was released during clotting *in vitro*. It may be assumed that in these patients there was no increased expression of VEGF-A due to ischemic conditions only. A possible mechanism responsible for this phenomenon is polymorphism in the promoter region of the VEGF gene and/or mutation of hypoxia-inducible factor (HIF). Hypoxia, via HIF-1, is a major player in VEGF expression at the transcriptional level [17]. Brogan et al. [18] established single base changes within the regulatory region of the VEGF gene. These data suggest that polymorphic changes may lead to differences in VEGF expression between individuals and could potentially contribute to a variety of pathological processes. It will be of great interest to investigate the clinical consequences of the disturbances of hypoxia-induced VEGF expression. An intensive further investigation to determine the functional significance of the observed effects may help to better understand the cardiovascular pathology.

In summary, these results suggest that increased VEGF concentration is not common in patients with STEMI since increased levels of the cytokine, both in plasma and serum, were seen in 17 of the 40 patients studied here. However, all the patients with SA exhibited elevated concentrations of VEGF-A in serum, but not in plasma. Moreover, it seems that platelets, but not ischemic or damaged myocardium, are the source of increased VEGF-A in the circulation. Whether the patients with elevated VEGF concentration exhibit a more efficient formation of a coronary collateral than patients with STEMI or SA in whom VEGF-A remains unchanged will require more detailed studies.

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