Experimental Gerontology 77 (2016) 19–28

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Occurrence of fibronectin–fibrin complexes in plasma of patients with multimorbidity due to the inflamm-aging phenomenon

Małgorzata Pupek [a,*], Robert Pawłowicz [b], Karolina Lindner [b], Dorota Krzyżanowska-Gołąb [a], Anna Lemańska-Perek [a], Bernard Panaszek [b], Iwona Kątnik-Prastowska [a]

[a] Department of Chemistry and Immunochemistry, Wrocław Medical University, Bujwida 44a, 50-345 Wrocław, Poland
[b] Department and Clinic of Internal Diseases, Geriatrics and Allergology, Wrocław Medical University, Curie-Skłodowskiej 66, 50-367 Wrocław, Poland

ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form 10 February 2016
Accepted 12 February 2016
Available online 13 February 2016

Section Editor: Chennai Guest Editor

Keywords:
Fibronectin
EDA-fibronectin
FN–fibrin complexes
FN agarose-immunoblotting
C-reactive protein
Multimorbidity

ABSTRACT

Background: Multimorbidity is the co-occurrence of chronic diseases associated with low-grade chronic inflammation of connective tissue.
Aim of study: Frequency of occurrence and relative amounts of fibronectin (FN) complexes with fibrin (FN–fibrin) and FN monomer were analyzed in 130 plasma samples of 18 to 94-year-old multimorbid patients in relation to concentrations of FN and extra domain A (EDA)-FN, and C-reactive protein (CRP) as well as to age, number of coexisting chronic diseases and presence of specified diseases.
Results: Immunoblotting revealed, besides FN dimer, the presence of FN monomer, and 750-, 1000-, and 1300-kDa FN–fibrin complexes in the multimorbid plasmas. The FN–fibrin complexes appeared more frequently and in higher relative amounts, but FN monomer less frequently and in a lower relative amount in the groups of elderly multimorbid patients, with a higher number of coexisting diseases and with dominance of cardiovascular diseases and osteoarthrosis, and with CRP concentration of 3–5 mg/l. In contrast, the normal plasma contained only the FN–fibrin complex of 750 kDa in a lower relative amount, but with an increasing amount with normal aging. Moreover, FN concentration increased and EDA-FN decreased with the number of co-existing diseases and aging of patients, although both concentration values were lower than in the age-matched normal groups. FN concentration was the lowest in the exacerbation of a chronic disease and EDA-FN in the stable chronic disease groups.
Conclusion: The alterations in plasma FN molecular status were associated with micro-inflammation and micro-coagulation, as well as multimorbidity of subjects and their physiological aging.

1. Introduction

Evidence from both developed and developing countries shows that older people are at high risk of multiple co-morbidities (Salisbury et al., 2011), i.e. the coexistence of additional diseases with an index disorder (Feinstein, 1970). The co-occurrence of two or more chronic conditions in one person, characterized by complex interactions of co-existing diseases where one is not necessarily more central than the others, is termed multimorbidity. It may represent the most common "disease pattern" found among the elderly, where a medical approach focused on a single disease does not suffice (van den Akker et al., 1996; Valderas et al., 2009).

Chronic inflammation, aging-related oxidative stress, increased posttranslational protein modifications and variable biochemical changes are a major underlying condition of many age-related diseases, such as metabolic syndrome, diabetes, cardio-vascular and lung system impairment, renal failure, osteoporosis, musculoskeletal dysfunction as well as mental disorders (Scharf et al., 2013; Chung et al., 2009). In the pathogenesis of several chronic diseases of the old age the most attractive hypothesis is the phenomenon of low-grade systemic inflammation. This phenomenon seems to be universal and is called inflamm-aging (Panaszek et al., 2009; Candore et al., 2010). In clinical practice, CRP concentration measurement is reported to have not only some definite value in evaluating clinical course and response to treatment in connective tissue disorders (Bassuk et al., 2004; de Ferrantia and Rifai, 2007), but also it is a useful marker of inflamm-aging (Ansar and Ghosh, 2013).

The low-grade systemic inflammation accompanied by several chronic age-dependent diseases may provoke destruction and remodeling of connective tissue and extracellular matrix (ECM) (Labat-Robert, 2012). Fibronectin (FN) is a multidomain and multifunctional glycoprotein present in ECM and cells, blood plasma and other biological fluids

* Corresponding author.
E-mail addresses: malgorzata.pupek@umed.wroc.pl (M. Pupek), robert.pawlowicz@umed.wroc.pl (R. Pawłowicz), karolina.lindner@umed.wroc.pl (K. Lindner), dorota.krzyzanowska-golab@umed.wroc.pl (D. Krzyżanowska-Gołąb), anna.lemanska-perek@umed.wroc.pl (A. Lemańska-Perek), bernard.panaszek@umed.wroc.pl (B. Panaszek), maria.katnik-prastowska@umed.wroc.pl (I. Kątnik-Prastowska).

http://dx.doi.org/10.1016/j.exger.2016.02.006
0531-5565/

(reviewed in To and Midwood, 2011). The basic FN form is a dimer composed of two 230–250-kDa polypeptides assembled into several domains with binding sites for ECM proteins (e.g., collagen, FN itself or FN fragment/s), cell surface receptors (integrins, bacterial FN receptors), blood protein derivatives (fibrin), and glycosaminoglycans (heparin) (To and Midwood, 2011). Additionally, the alternatively spliced extra domains A (EDA), B (EDB), and oncofetal IIICS region may or may not be included in the FN polypeptide sequences, altering the structure, solubility and function of FNs (White et al., 2008).

Produced by the liver, FN is released to the blood stream as a compact globular plasma FN (pFN) lacking EDA and EDB domains (White et al., 2008; To and Midwood, 2011) and circulates in the blood at a relatively high concentration of about 280 mg/l (Lemańska-Perek et al., 2013). The pFN can readily form aggregative amyloid-like structures triggered by exposed structures (Pellenc et al., 2006), and can act as a non-specific opsonin which facilitates the removal by macrophages and neutrophils of tissue debris containing collagen, fibrin, bacteria, and other insoluble molecules (Hynes and Yamada, 1982). Moreover, pFN is reported to be a reservoir for tissue needs (Moretti et al., 2007), and being transferred from blood to tissue participates in ECM remodeling, tissue repair during endothelial injury and fibrin clot formation, playing an essential role in fibrillogenesis and modulation of hemostasis (To and Midwood, 2011; Yi et al., 2003; Moretti et al., 2007; To and Midwood, 2011). Plasma FN is reported to prevent platelet aggregation and thrombus formation. In contrast, pFN in the presence of fibrin supports platelet aggregation and thrombus growth and stability (Wang et al., 2014; Wang and Ni, 2015). Wang and Ni (2015) suggested that the pFN–fibrin complex (FN–fibrin) may be a prerequisite for its prothrombotic activities.

Cellular FN (cFN) synthesized locally by different cell types (i.e. hepatocytes, endothelial cells, fibroblasts, macrophages, lymphocytes, platelets, chondrocytes, myocytes and tumor cells) contains variable proportions of the EDA and EDB domains and is normally found in plasma in relatively low amounts (Chauhan et al., 2008; White et al., 2008). Cellular FN is essential in cell adhesion ensuring cohesion between different ECM macromolecules, or cells and ECM during embryogenesis, remodeling of ECM, tissue repair, tumor progression, and inflammation (Labat-Robert, 2001; White et al., 2008; Maurer et al., 2010; To and Midwood, 2011). EDA-FN and platelet TLR4 interaction promotes arterial thrombosis, suggesting a possible link between elevated EDA-FN, innate immunity, and thrombotic risk (Prakash et al., 2015; Wang and Ni, 2015).

FN is reported to undergo destructive modifications with aging and/or by pathological events accompanied to age-related diseases. During normal biological aging that is mostly complicated by coexisted polypathologies (Barodka et al., 2011; Labat-Robert, 2001; Fabbri et al., 2015) the FN molecule can undergo structural changes, glycoxidation, fragmentation, and cross-linking among fragments and intact FN (Antia et al., 2008; Sakata et al., 2000; Labat-Robert, 2001; Scharf et al., 2013). Such modifications can lead to a decrease of FN's ability to bind to other matrix components, and/or reveal buried epitopes, making them free for the reaction with respective ligands (To and Midwood, 2011; Lu et al., 2011). Our group has reported the presence of atypical high-molecular FN-reduced form of 280 kDa in plasma of patients with Alzheimer's disease (Lemańska-Perek et al., 2009), lung cancer (Pupek et al., 2009), and aged individuals (Lemańska-Perek et al., 2013), which were generally absent in normal human plasma of young and middle age individuals (Lemańska-Perek et al., 2013).

The use of immunoblotting with anti-fibronectin and anti-fibrinogen antibodies after SDS-agarose-gel electrophoresis under non-reducing conditions allowed us to separate a series of bands showing molecular masses of 750 kDa and higher, which were identified as FN complexes with fibrin (FN–fibrin) composed also from the 280-kDa FN subunit (Krzyżanowska-Gołąb et al., 2014). The FN–fibrin complex/es were observed in plasma of patients suffering from inflammatory diseases (Krzyżanowska-Gołąb et al., 2014; Lemańska-Perek et al., 2015).

In the present study frequency of occurrence and relative amounts of FN–fibrin complexes were analyzed in plasma of patients with multimorbidity due to the inflamm-aging phenomenon and with respect to FN and EDA-FN concentrations, occurrence of FN monomer, and eventual presence of FN fragments. The FN and EDA-FN concentrations were estimated by ELISA with two specific monoclonal antibodies, and occurrence and relative amounts of FN–fibrin complexes and FN monomer as well as the presence of any FN degradation products were analyzed by SDS-agarose immunoblotting and Western blotting in plasma of patients with multiple chronic conditions. The data were related to: 1) patient's age, 2) number of diseases the patients suffered from, 3) concentration of C-reactive protein (CRP) in patient's plasma as an indicator of low-grade systemic (<5 mg/l) and chronic inflammation (>5 mg/l). We suspected that determination of the plasma FN molecular status in the context of multimorbid complexity may help in understanding the multidirectional role of FN in human aging.

2. Material and methods

2.1. Patients

Patients (n = 130) attending the Department and Clinic of Internal Diseases, Geriatrics and Allergology of Wroclaw Medical University who suffered from multiple chronic diseases (multimorbidity) were included in the study after their informed consent had been given. The study was approved by the local Commission of Bioethics at Wroclaw Medical University, approval no. KB-445/2012.

The male (n = 42) and female (n = 88) patients aged from 18 to 94 years (n = 130; 66.5 ± 16.9 years) underwent a clinical examination including general status as well as medications. Patients were included if they were adults and suffered from two or more co-existing chronic diseases. Exclusion criteria were 1) one acute or chronic disease, 2) cancer, 3) pregnant or lactating women 4) substance abuse. Multimorbidity was defined as the co-occurrence of two or more chronic diseases within one person (van den Akker et al., 1996; Valderas et al., 2009).

The individuals included in the study suffered from 2 to 11 diseases. Overall, a total of 25 chronic diseases occurred in this group The most frequent diseases (number, percentage of patients) were: hypertension (90, 69.2%); cardiac diseases (ischemic heart disease, heart failure, cardiac dysrhythmia; 75, 57.7%); atherosclerosis (66, 50.8%); osteoarthrosis (55, 42.3%); lipid disorders (51, 39.2%); chronic inflammation (46, 35.4%); allergy (atopic asthma, allergic rhinitis, anaphylaxis, acute or chronic urticaria, drug allergy, insect venom allergy; 43, 33.1%); cerebrovascular diseases (stroke and transient ischemic attack, vascular dementias, depression, anxiety disorders; 40; 30.8%); asthma (37, 28.5%); digestive system diseases (chronic gastroduodenitis, esophageal reflux disease, diverticular disease; 36, 27.7%); diabetes mellitus (32, 24.6%); anemia (26, 20%); thyroid disease (23, 17.7%); varicose veins (22, 16.7%); liver disease (fatty liver disease, cirrhosis, toxic and drug-induced liver injury; 21, 16.2%); chronic obstructive pulmonary disease, COPD (17, 13.1%); renal failure (16, 12.3%); cholelithiasis (16; 12.3%); osteoporosis (15, 11.5%); deep vein thrombosis (9, 6.9%); and 20 (15.4%) patients suffered from some benign tumors such as: benign prostatic hyperplasia (7), gallbladder polyps (2), uterine fibroids (uterine leiomyoma (2), and single cases of thyroid nodule, angiomyolipoma of kidney, meningioma, osteoma, spinal cavernous angioma (hemangioma of spine), benign adrenal tumor, ovarian cyst, cyst of medulla of kidney, esophageal polyp, and solitary pulmonary nodule. Nephrolithiasis, autoimmune diseases, pancreatitis, and skin diseases represented less than 5% of patients each.

The multimorbid patients' samples were divided into three different groups according to: 1) the patient's age and 2) number of chronic diseases, 3) concentration of plasma CRP and patient's condition.

Based on the patient's age, three subgroups 1a–1c were specified: 1a, Multimorbid adults (n = 13; aged from 18 to 41 years, 30.3 ± 7.6 years; 6 men and 7 women), 1b, Multimorbid middle age (n = 42; aged from