The role of fractalkine (FKN, CX3CL1) in the pathogenesis of insulin resistance and type 2 diabetes

Rola fraktalkiny (FKN, CX3CL1) w patogenezie insulinooporności i cukrzycy typu 2

Katarzyna Bergmann

Katedra i Zakład Diagnostyki Laboratoryjnej, Collegium Medicum UMK im. L. Rydygiera w Bydgoszczy

Summary
Earlier studies particularly underline the relationship between adipose tissue dysfunction and the risk of type 2 diabetes, therefore the search for new biomarkers of insulin resistance, especially those produced by adipocytes is a challenge for contemporary biochemical sciences. The aim of this review was to describe recently discovered adipocytokine: fractalkine (CX3CL1, FKN) as a potential marker of pancreatic β cells dysfunction which links low-grade inflammation in adipose tissue with impaired glucose metabolism and might indicate new diagnostic and therapeutic targets.

Streszczenie
Badania z ostatnich kilkunastu lat w sposób szczególny zwracają uwagę na zależności między dysfunkcją tkanki tłuszczowej a ryzykiem rozwoju cukrzycy typu 2, dlatego poszukiwanie nowych biomarkerów insulinooporności, zwłaszcza tych produkowanych przez adipocyty stanowi spore wyzwanie. Celem niniejszej pracy było przedstawienie odkrytej niedawno adipocytokiny: fraktalkiny (FKN, CX3CL1) jako potencjalnego markera dysfunkcji komórek β trzustki, który łączy stan zapalny o niskim nasileniu w tkance tłuszczowej i zaburzenia metabolizmu glukozy, wyznaczając tym samym nowe cele diagnostyczne i terapeutyczne.

Key words: adipocytokines, diabetes, fractalkine, insulin resistance

Słowa kluczowe: adipocytokiny, cukrzyca, fraktalkina, insulinooporność

Introduction
Type 2 diabetes (T2DM) mellitus is one of the most important health and socio-economic problems of developed countries, however in recent years a significant increase of morbidity in developing countries has been observed. According to the International Diabetes Federation (IDF) in 2035 diabetes will affect one out of ten adults, which means that the incidence of disease will rise up to 592 millions worldwide [1]. Due to the chronic nature of diabetes and the initial lack of characteristic symptoms, there is still a high proportion of undiagnosed patients. Therefore the need to explore for new risk factors and potential biomarkers of early insulin resistance are the main areas of interest for contemporary biochemistry and laboratory medicine.

Pathogenesis of T2DM is closely related to adipose tissue dysfunction and low-grade inflammation. Excessive body fat acts as an endocrine organ which produces specific cytokines (adipocytokines) such as tumor necrosis factor α (TNF-α), interleukin 1β (IL-1 β ), interleukin 6 (IL-6), resistin, retinol binding protein 4 (RBP-4), dipeptidyl peptidase 4 (DPP-4) and adipocyte fatty-binding protein (A-FABP). In consequence activation of inflammation pathways affects genes expression, decreases the quantity/activity of the insulin-dependent receptors and insulin production in β-cells, leading to insulin resistance [2, 3]. Although diagnostic and clinical utility/relevance of most cytokines are not clearly confirmed yet, a number of studies indicate that elevated levels of various chemokines may predict the occurrence of insulin resistance and diabetes even within several years. Recently discovered chemokine: fractalkine (FKN, CX3CL1) is considered as a promising biomarker of early pancreatic β-cells dysfunction in both apparently healthy, non-diabetic and overweight/obese subjects. Characteristics of fraktalkine in table I.

Fractalkine – structure and biological activity
Chemokine (C-X3-C motif) ligand 1 (CX3CL1) is a protein composed of 373 amino acids and the only known member of the CX3C chemokine family. In humans it is commonly known under the name fractalkine (FKN), while in mice it
The role of fractalkine (FKN, CX3CL1) in the pathogenesis of insulin resistance and type 2 diabetes

is usually called neurotactin. The polypeptide structure of CX3CL1 differs from the typical structure of other chemokines. Fractalkine is synthesized as a transmembrane molecule consisted of a soluble form (chemokine domain and the extracellular mucin-like stalk) and the membrane-bound form (Fig. 1). Generation of the soluble FKN depends on disintegrin-like metaloproteinases (ADAM) 10 and 17/TACE. The basic property of this molecule is chemoattractive activity for monocytes, natural killer (NK) cells and T lymphocytes. Membrane-bound form is produced in various cells, e.g. vascular smooth muscle cells, endothelial and epithelial cells, dendritic cells after stimulation by proinflammatory factors (TNF-α, IFN-γ, IL-1). It promotes strong adhesion of leukocytes to activated endothelial cells, where it is primarily expressed [5]. The expression of FKN mRNA is observed in various organs such as heart, kidneys, brain, liver, adrenal gland and adipocytes. CX3CL1 signals trough a single Gαi-linked receptor CX3CR1. This receptor occurs on the surface of monocytes, macrophages, NK cells, some T cells, neutrophils, mast cells, platelets and smooth muscle cells. Low expression of CXCR1 on cell surface corresponds to CD14− and CD16+ monocyte subset, while high expression is related to CD14+ and CD16− monocyte populations in humans [6].

The classical pathway of cell adhesion and migration through the endothelium assumed that this process is dependent on the selectin-mediated interactions between leukocytes and the endothelium and activation of integrins on leukocytes by chemokines presented on glycosaminoglycans. Chemokines were considered as soluble molecules associated with proteoglycans on the cell surface and tissue matrix [7]. By the discovery of specific properties, fractalkine changed the existing opinions. Chemokine domain of FKN on the top of the mucin-like stalk acts as an adhesion molecule involved in all stages of the migration, while the soluble form shows strong chemotactic effect [8]. CX3CR1 receptor on leukocytes connects selectively with a chemokine domain presented on endothelial cells which triggers rapid binding. Interaction of FKN with CX3CR1 additionally increases expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and integrins in leukocytes [8], which may result in stronger adhesion. Expression of fractalkine attracts and activates NK cells which are responsible for cytotoxic effect and cytolysis, therefore its contribution to inflammation-dependent conditions seems to be very important [9].

**Relevance of fractalkine in adipose tissue inflammation and insulin resistance**

Several studies confirmed the role of fractalkine in the pathogenesis of many human pathologies, such as atherosclerosis and cardiovascular disease, rheumatoid arthritis, HIV infections and allograft rejection in which activity of inflammatory factors is significant [5, 8, 10, 11]. Type 2 diabetes is strictly associated with excessive adipose tissue and chronic low-grade inflammation in adipocytes. A key role in the early stages of insulin resistance development is played by the recruitment of monocytes to adipose tissue which initiates local production of pro-inflammatory cytokines. Chronic inflammation developing as a consequence of increased circulating cytokines induces insulin resistance. Dual properties of FKN in the mechanism of leukocytes chemotaxis and adhesion might be a relevant pathogenetic factor in early adipocytes dysfunction and T2DM [12], although studies in this area are limited. The proinflammatory feature of fractalkine in adipose tissue was indirectly confirmed in study by Digby et al. [13] in which FKN activity was decreased by poten-
tial anti-inflammatory factor, nicotinic acid, in cultured 3T3-L1 adipocytes. Results showed that TNF-alpha treatment (1.0 ng/mL) of adipocytes increased gene expression of fractalkine (9±3.3-fold; p<0.01) and also other inflammation-related factors, such as monocyte chemoattractant protein-1 (MCP-1) and inducible nitric oxide synthase (iNOS). The addition of nicotinic acid (10^{-4} M) to adipocytes attenuated expression of fractalkine (50±12%; p<0.01) and this state was mirrored in protein released from the adipocytes into the surrounding media. The association of fractalkine expression with inflammation induced by controlled low-dose endotoxemia in human adipocytes was observed by Mehta et al. [14]. In this study CX3CL1 mRNA increased 15-fold (p<0.001) after 4 hours of LPS administration (0.6 ng/kg) and it was the largest increase compared to the other cytokines (IL-6, TNF-α and MCP-1). In LPS-treated subjects compared to placebo a significant 32% increase in HOMA-IR and 21% decreased in insulin sensitivity (p<0.05) was observed.

Study by Shah et al. [15] clearly confirmed expression and secretion of CX3CL1 in human adipocytes, therefore it can be considered as adipocytokine. This well-design study was performed based on three types of tests: 1) in vivo endotoxemia and adipose tissue biopsies in lean and obese subjects; 2) in vitro study of primary human adipocytes and monocytes; 3) case-control study in individuals with and without T2DM. Significantly increased adipose and blood FKN levels (33-fold; p<0.001 and over 40-fold; p=0.006, respectively) were observed after 4 hours of administration of moderate dose (3 ng/kg) of standard lipopolysaccharide (LPS) endotoxin. Levels of CX3CL1 in subcutaneous adipose tissue were significantly higher in obese compared with lean subjects (mean 0.420 vs. 0.228 ng/mL; p=0.04). Moreover, in obese individuals FKN in subcutaneous adipose tissue was lower than in visceral adipose tissue (mean 0.420 vs. 0.736 ng/mL; p=0.01). Monocyte adhesion to adipocytes was reduced almost half after dosing of CX3CL1 blocking antibody. Plasma FKN was significantly higher in T2DM patients compared to controls (mean 0.506 vs. 0.422 ng/mL; p<0.0001). After adjusting for gender, BMI, ethnicity and other metabolic risk factors a significant 2.77 odds ratio for diabetes for a 1 SD (0.21 ng/mL) increase in fractalkine concentration was observed. Presented results highlight the influence of fractalkine-mediated pathway on adipose tissue inflammation, however do not explain the effect of FKN on β-cells function.

Recent study by Lee et al. [16] demonstrate that fractalkine/CX3CR1 system regulates in vitro pancreatic β-cell function by improving insulin secretion and glucose uptake in mouse and human pancreatic islets. Authors explained described hypothesis with three mechanisms: 1) anti-apoptotic effect via increased expression of Akt-1 kinase, 2) increased intracellular Ca^{2+} levels which stimulates insulin secretion and 3) suppression of inducible cAMP early repressor (ICER-1) and maintenance of β-cells gene expression. In vivo administration of FKN to CX3CR1 knockout mice improved glucose tolerance with an increase in insulin secretion. Moreover, expression of FKN in islets was decreased by high-fat diet, obesity and aging. In conclusion authors suggest that lower levels of CX3CL1/CX3CR1 may contribute to β-cell dysfunction in T2DM and indicate its potential role as a new therapeutic target. However, these findings seem to be controversial when they are compared with results of Wan et al. [17], which showed suppressed FKN expression and CX3CL1/CX3CR1 signaling by rosiglitazone, a drug for insulin resistance and type 2 diabetes. Rosiglitazone acts by activation of peroxisome proliferator-activated receptor γ (PPAR-γ), an anti-inflammatory agent that inhibits FKN-dependent migration and adhesion of macrophages to endothelial cells. Similar effects were obtained in obese T2DM patients treated with pioglitazone, which decreased circulating levels of fractalkine, IL-6 and MCP-1 and improved glucose metabolism due to the same mechanism as rosiglitazone [18]. It is worth noting that several studies revealed the role of increased FKN levels in atherosclerosis and vascular disorders in T2DM [19, 20]. Moreover, high glucose conditions might induce upregulation of fractalkine and MCP-1 in smooth muscle cells [21], as well as upregulation of fractalkine and P-selectin and increased monocyte adhesion in human endothelial cells [22]. The probable mechanism depends on increase of NADPH oxidase activity and generation of intracellular reactive oxygen species which activates the NF-κB and AP-1 transcription factors and enhances production of pro-inflammatory cytokines. Taking into account all these findings it can be concluded that initial increase of insulin secretion which has a well established anti-inflammatory activity [23] by CX3CL1/CX3CR1 system might be a defensive reaction to inflammation which can lead to chronic hyperinsulinaemia and dysfunction of β-cells.

Conclusions

Discovery of new potential biomarkers of insulin resistance and diabetes related to adipocytes and low-grade inflammation may provide an opportunity for the development of new therapeutic targets. Despite the promising results on the contribution of fractalkine in the pathogenesis of β-cells dysfunction, more detailed large population-based studies to evaluate their clinical and diagnostic utility are required.

References:

5. Owlsíniak P, Zajkowska JM, Pietruczuk M, et al. Fractalkine -