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PRACA ORYGINALNA
ORIGINAL PAPER

Subtilisin/kexin type 9 protein convertase and interleukin 1 beta alterations in acute myocardial infarction

Zmiany ekspresji konwertazy białkowej subtylizyny/keksyny typu 9 oraz interleukiny 1 beta w ostrym zawale serca

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ABSTRACT

Lipids and inflammation are crucial components in acute myocardial infarction (AMI) pathophysiology. The aim of this study was to assess genetic expression of interleukin 1 beta (IL-1β) and subtilisin/kexin type 9 protein convertase (PCSK9) in AMI patients. 112 AMI patients – 55 with ST-segment elevation myocardial infarction (STEMI) and 57 with non-ST-segment elevation myocardial infarction (NSTEMI) – aged 35 to 92 (average age 65) were enrolled into the study. Control subjects were those with excluded coronary artery disease (CAD; n = 41) and with chronic coronary syndrome (CCS; n = 53). RNA extraction from peripheral blood mononuclear cells (PBMCs) using TRIzol Reagent (Invitrogen) method and genetic expression using quantitative real-time polymerase chain reaction (QRT PCR) method were performed. PCSK9 expression was higher ($p = 0.04$) and IL-1β lower ($p < 0.001$) in AMI subjects compared to controls. Higher PCSK9 transcriptional activity was found in more advanced stages of CAD, in male, in cases of increased body weight, decreased left ventricular ejection fraction (LVEF), and high-density lipoprotein (HDL) cholesterol concentration. Higher IL-1β expression was observed in patients with AMI and concomitant hypercholesterolemia. Thorough understanding of IL-1β and PCSK9 biology, key representatives of two basic pathophysiological links underlying myocardial infarction, is of great practical importance. This is particularly important due to currently wide availability of pharmacological intervention within metabolic pathways of these molecules.

KEYWORDS

protein convertase subtilisin/kexin type 9, interleukin 1 beta, acute myocardial infarction, gene expression, peripheral mononuclear blood cells

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STRESZCZENIE

Lipidy oraz zapalenie stanowią dwa fundamentalne ogniwa w patofizjologii ostrego zawału serca (*acute myocardial infarction* – AMI). Celem niniejszej pracy była ocena ekspresji genów interleukiny 1 beta (IL-1β) oraz konwertazy białkowej subtylizyny/keksyny typu 9 (*proprotein convertase subtilisin/kexin type 9* – PCSK9) u chorych z AMI. Do badania włączono 112 hospitalizowanych chorych z AMI, spełniających kryteria włączenia i wyłączenia – 55 z zawałem serca z uniesieniem odcinka ST (STEMI) oraz 57 z zawałem serca bez uniesienia odcinka ST (NSTEMI) – w wieku od 35 do 92 lat (średni wiek 65 lat). Grupę kontrolną stanowiły osoby z wykluczoną w koronarografii chorobą wieńcową (*coronary artery disease* – CAD; n = 41) oraz osoby z przewlekłym zespołem wieńcowym (*chronic coronary syndrome* – CCS; n = 53). Materiał RNA z komórek jednojądrzastych krwi obwodowej (*peripheral blood mononuclear cells* – PBMCs) uzyskano za pomocą metody TRIzol Reagent (Invitrogen), a ocenę ilościową ekspresji genów oceniano za pomocą metody reakcji łańcuchowej polimerazy w czasie rzeczywistym (*quantitative real-time polymerase chain reaction* – QRT PCR). Ekspresja PCSK9 była większa ($p = 0,04$), a IL-1β mniejsza ($p < 0,001$) u chorych z AMI w porównaniu z grupą kontrolną. Większą aktywność transkrypcyjną PCSK9 stwierdzono w bardziej zaawansowanych postaciach CAD, u mężczyzn oraz w przypadkach zwiększonej masy ciała, niższej frakcji wyrzutowej lewej komory (*left ventricular ejection fraction* – LVEF) i niższego stężenia cholesterolu frakcji HDL (*high-density lipoprotein*). Z kolei większą ekspresję IL-1β obserwowano u chorych z AMI i współistniejącą hipercholesterolemią. Dokładne zrozumienie biologii IL-1β i PCSK9, dwóch reprezentantów kluczowych ogniw patofizjologicznych w zawale serca, ma duże znaczenie praktyczne, szczególnie w kontekście szeroko aktualnie dostępnych metod farmakologicznej interwencji w ich szlaki metaboliczne.

SŁOWA KLUCZOWE

konwertaza białkowa subtylizyny/keksyny typu 9, interleukina 1 beta, ostry zawał serca, ekspresja genów, jednojądrzaste komórki krwi obwodowej

INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) remains leading cause of morbidity and mortality in developed countries [1]. According to the most popular hypothesis, atherosclerosis is a chronic inflammatory process constituting a defensive response of vascular wall to damaging factors, especially hypercholesterolemia with the pivotal role of low--density lipoproteins (LDLs) [2]. Links between lipid metabolism and inflammation, key determinants in the pathophysiology of atherosclerosis, which is the main cause of acute myocardial infarction (AMI), are very complex and not fully understood. This paper aims to assess key molecules of these pathophysiological links, interleukin 1 beta (IL-1β) and subtilisin/kexin type 9 protein convertase (PCSK9), gene expression in AMI patients' peripheral mononuclear cells.

PCSK9 plays crucial role in the regulation of cholesterol homeostasis. It is a serine protease and ligand for the LDL receptor (LDLR). LDL binds to LDLR and is internalized being removed from circulation. It is then digested in lysosomes and LDLR is re-cycled to the surface, where continue its LDLs clearance. PCSK9 leads to lysosomal degradation of LDLR resulting in its decreased surface expression. It also inhibits recirculation of LDLR in hepatocytes. Abovementioned mechanisms result in lower LDLR expression and increased LDLs levels [3].

Inflammatory pathways drive atherosclerosis and are possible connection between conventional cardiovascular risk factors, atherosclerosis and its complications. The pivotal proinflammatory cytokine is IL-1β. It has become, in numerous clinical trials, the target of interventions aimed at inhibiting its action. Intracellular protein complex (NLR family pyrin domain containing 3 – NLRP3) forms a macromolecular structure called the NLRP3 inflammasome. Increased expression of NLRP3 inflammasome genes in human atherosclerotic plaques and their correlation with the severity of coronary artery disease (CAD) has been well proven [4,5].

Inflammation results in significant lipid and lipoprotein metabolism changes [6]. By reducing LDLR mRNA leads to an increase in very low (VLDLs) and LDLs and decrease of high-density lipoproteins (HDLs) cholesterol serum concentrations. Such a process has been well documented in an animal model with the use of lipopolysaccharide, which is often used as a model of infection and inflammation [7]. Moreover, it has been shown that reduced concentration of PCSK9 is associated with increased lipid clearance of pathogens by LDLR, reduced inflammatory response and better prognosis in patients with septic shock [8]. It was also shown that PCSK9 is present in atherosclerotic plaque and released from smooth muscle cells it is involved in the expression of LDLR in macrophages [9]. There is also some evidence for direct PCSK9 induction by inflammation [10].

Available data concern mainly the concentrations of studied molecules, which is not a simple, linear derivative of genetic expression. There is less data on gene expression itself, which is a very complex process. There are some data showing different expression and concentration levels of LDLR influenced by inflammatory process [11]. Links between the number

of gene transcripts and lipid profile are also unclear. The aim of the study was to assess mRNA levels of IL-1β and PCSK9 in peripheral blood mononuclear cells (PBMCs) of patients with AMI.

MATERIAL AND METHODS

The study protocol was approved by the Ethics Committee of Medical University of Silesia in Katowice (approval No. KNW/0022/KBI/98/15). All procedures were performed in accordance with the ethical standards formulated in the Helsinki Declaration. All participants have signed the informed consent.

112 patients, meeting the inclusion and exclusion criteria, admitted to the Department of Cardiology diagnosed with AMI (ST-segment elevation myocardial infarction – STEMI n = 55 and non-ST-segment elevation myocardial infarction – NSTEMI n = 57) were enrolled into the study. The inclusion criteria were: typical angina in the last 24 hours preceding admission to the hospital, abnormal electrocardiogram (ECG), biochemical evidence for cardiac necrosis, and CAD confirmed in coronary angiography. The exclusion criteria were: ongoing use of statins, lack of informed consent, previous severe heart failure (left ventricular ejection fraction – LVEF < 35%), malignancy, active infection, contact difficulties (stroke, mental disorders), and severe renal failure (estimated glomerular filtration rate – eGFR < 30 ml/min/m^2). Control group involved 53 patients with chronic coronary syndrome (CCS) and 41 subjects with excluded CAD, in whom no changes on coronary angiography were visualized (i.e. high risk occupation, myocardial bridge). Within 24 hours from admission, a detailed medical history was taken and following procedures were performed: physical examination, standard 12-lead ECG, echocardiography as well as blood samples were taken for biochemical and molecular analyses. Biochemical tests included: blood count cell, serum levels of electrolytes, creatinine, glucose, lipid profile and markers of myocardial necrosis: cardiac troponin T and creatine kinase MB (CK-MB) isoenzyme.

Material for molecular tests was peripheral venous blood. The research was carried out on easily available population of PBMCs, which is of key importance in the inflammatory process – lymphocytes and monocytes. RNA extraction was performed using TRIzol Reagent (Invitrogen). Evaluation of genetic expression of studied genes was performed using the quantitative real-time polymerase chain reaction (QRT PCR) method. Expression of studied genes was inferred from the mRNA copy number per 1 μg of total RNA.

Statistical analysis was performed using MedCalc and Statistica v.12.0 software. For quantitative variables number, arithmetic means, minimum and maximum value, and standard deviations (SD) of the estimated parameters were calculated. Qualitative variables were analyzed by calculating number and percentage of each value. The Shapiro-Wilk test was used to test the distribution of analyzed variables. Because the analyzed variables significantly differed from the normal distribution, non-parametric the Mann-Whitney U and Kruskal-Wallis tests were applied. Frequency analysis was conducted using Fisher's exact test or χ^2 test. The non-parametric Spearman correlation coefficient was used to analyze the correlation between selected parameters. A p-value of 0.05 or below was considered statistically significant.

RESULTS

General characteristics

Characteristics of the study group, taking into account demographic data, cardiovascular risk factors, and comorbidities is presented in Table I.

Majority of study group were men. Patients with myocardial infarction (MI) were more often current smokers and had positive family history for CAD compared to controls. They were also less frequent obese. There were no statistically significant differences between STEMI and NSTEMI individuals regarding demographic data, cardiovascular risk factors, and comorbidities occurrence.

The results of laboratory tests in the studied group are presented in Table II.

Table I. Characteristics of studied population of patients with acute myocardial infarction and controls including demographic data, cardiovascular risk factors and comorbidities

Entire studied group n = 206							
Parameter n; % of subgroup	MI n = 112 (100%)		CCS n = 53 (100%)		non-CAD n = 41 (100%)		p-value
Age [yrs] range mean	32–92 $\bar{x}$ 63.88		48–84 $\bar{x}$ 67.83		26–77 $\bar{x}$ 53.61		**0.004**
	number [n]	percentage [%]	number [n]	percentage [%]	number [n]	percentage [%]	
Sex	W = 32	28.6	W = 19	35.8	W = 19	46.3	0.114
Cardiovascular risk factors							
FH	29	26	5	9.4	6	14.6	**0.03**
Current smokers	47	42	9	17	3	7.3	**< 0.005**
Ex-smokers	37	33	22	41.5	15	36.6	0.567
Obesity	27	24	37	69.8	18	43.9	**0.002**
Comorbidities							
AH	86	77	46	86.8	25	61	**0.014**
DM/IFG/IGT	42	37.5	26	49	10	24.4	**0.049**
PAD	11	10	9	17	0	0	**0.029**
AF	13	12	16	30	8	19.5	0.104
CAD	28	25	41	77	0	0	**< 0.005**

MI – myocardial infarction; CCS – chronic coronary syndrome; non-CAD – patients without coronary artery disease; W – women; FH – family history positive for cardiovascular disease; AH – arterial hypertension; DM – diabetes mellitus; IFG – impaired fasting glycaemia; IGT – impaired glucose tolerance; PAD – peripheral artery disease; AF – atrial fibrillation; CAD – coronary artery disease history.

Table II. Results of laboratory tests in studied group

Parameter	non-CAD n = 41		MI n = 112		CCS n = 53		p-value
	$\bar{x}$	± SD	$\bar{x}$	± SD	$\bar{x}$	± SD	
Total cholesterol [mg/dl]	152.38	32.15	189.34	47.97	171.33	46.30	**0.026**
HDL cholesterol [mg/dl]	47.25	16.68	40.52	12.4	52.13	25.32	0.0569
LDL cholesterol [mg/dl]	76.75	28.79	120.91	46.88	93.67	39.45	**0.001**
Triglycerides [mg/dl]	143	50.72	150.0	69.86	135.75	96.49	0.141
Troponin T on admission [ng/ml]	0.0088	0.004	0.56	1.20	0.015	0.009	**< 0.001**
Troponin T – control [ng/ml]	0.0045	0.002	1.52	1.20	0.015	0.09	**< 0.001**
Glucose [mg/dl]	104.78	21.28	140.91	59.81	114.76	35.23	**< 0.001**
Creatinine [mg/dl]	0.96	0.22	0.93	0.26	0.96	0.31	0.870

n – number; $\bar{x}$ – average; SD – standard deviation; non-CAD – patients without coronary artery disease; MI – myocardial infarction; CCS – chronic coronary syndrome; HDL – high-density lipoprotein; LDL – low-density lipoprotein.

In MI patients higher values of total cholesterol and LDLs were found, as well as lower levels of HDLs cholesterol compared to individuals with CCS and those with excluded CAD. There was also higher serum glucose concentration.

In STEMI compared to NSTEMI slightly higher glucose level was observed (143 mg/dl vs 131 mg/dl; p = 0.004). No other significant differences were observed in basic laboratory tests in the group of patients with AMI, taking into account the type of diagnosed MI (STEMI vs NSTEMI).

PCSK9 and IL-1β expression

IL-1β and PCSK9 gene expression in studied group are shown in Figure 1.

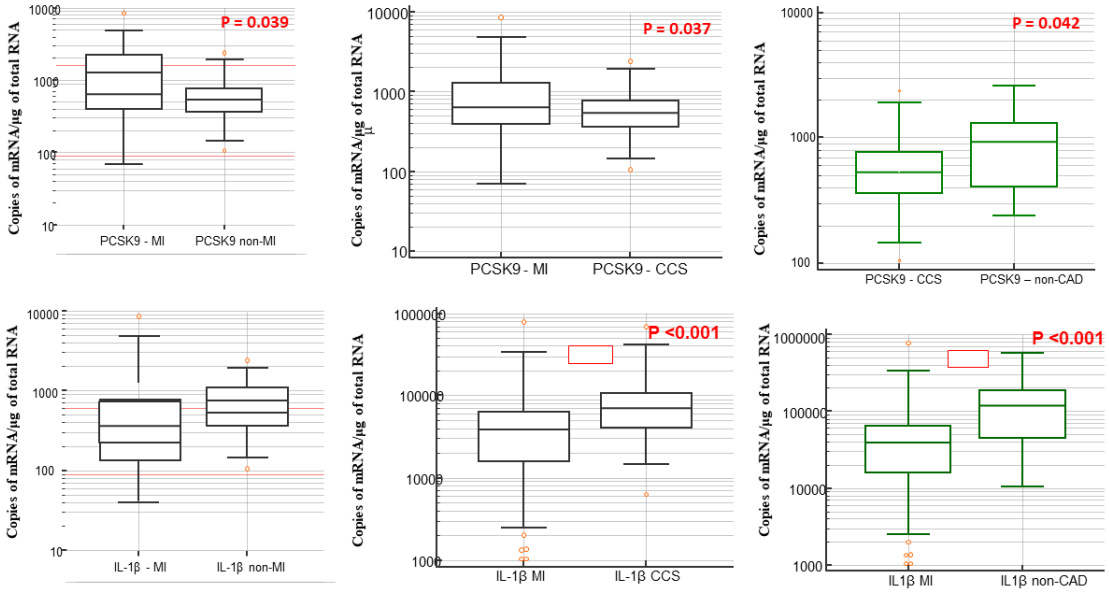

Fig. 1. Subtilisin/kexin type 9 protein convertase and interleukin 1 beta gene expression in patients with coronary artery disease. MI – myocardial infarction; CCS – chronic coronary syndrome; non-CAD – excluded coronary artery disease; PCSK9 – subtilisin/kexin type 9 protein convertase; IL-1β – interleukin 1 beta. Data analyzed using the Mann-Whitney U test.

AMI patients had higher expression of PCSK9 gene compared to the patients with CCS and those with excluded CAD (p = 0.037 and p = 0.042 respectively; Mann-Whitney U test). In turn, expression of IL-1β gene was lower in the AMI group compared to the group with stable form of CAD (p < 0.001) as well as those in whom CAD was excluded (p < 0.001). There were no significant differences in both, PCSK9 and IL-1β, gene expression regarding type of MI – STEMI vs NSTEMI (p = 0.1881 and p = 0.6858 for PCSK9 and IL-1β respectively).

In patients with AMI number of significant artery narrowings was related to higher PCSK9 gene expression (p = 0.038), but no such relationship was found in case of IL-1β (p = 0.416). Significantly higher PCSK9 mRNA level was found within patients burdened with poor prognosis factors, such as: male sex (p = 0.006), increased body weight (p = 0.05), reduced HDL cholesterol concentration (p = 0.04), and reduced LVEF (p = 0.210; ns).

Abnormal lipid profile was associated with increased expression of IL-1β gene in patients with MI compared to the controls (p = 0.025). Results of genetic expression analysis of IL-1β and PCSK9 in patients with MI, taking into account inappropriate lipid profile according to the current guidelines of the European Society of Cardiology on the management of dyslipidemias (total cholesterol > 190 mg/dl and/or LDL > 55 mg/dl and/or HDL < 45 mg/dl in women and < 40 mg/dl in men and/or triglycerides > 150 mg/dl) [12] are presented in Table III. The subjects belonged to the group of high or very high cardiovascular risk categories.

Within AMI patients elevated triglycerides and total cholesterol levels were connected with increased IL-1β expression (p = 0.027 and p = 0.056, respectively). PCSK9 expression was higher among men with lower HDL concentration (p = 0.044). Increased expression of both molecules – IL-1β and PCSK9 was demonstrated in cases of atherogenic dyslipidemia. These results are presented in Figure 2.

Table III. Subtilisin/kexin type 9 protein convertase and interleukin 1 beta genetic expression in studied group of patients regarding coexistence of abnormal lipid profile

Parameter	Subgroup	PCSK9	IL-1β
Triglycerides	MI_TG_n vs MI_TG_a	0.320	0.027
Total cholesterol	MI_TC_n vs MI_TC_a	0.488	0.056
HDL (women)	MI_HDL_W_n vs MI_HDL_W_a	0.807	0.387
HDL (men)	MI_HDL_M_n vs MI_HDL_M_a	0.044	0.940
LDL	MI_LDL_n vs MI_LDL_a	0.834	0.066

PCSK9 – subtilisin/kexin type 9 protein convertase; IL-1β – interleukin 1 beta; HDL – high-density lipoprotein; LDL – low-density lipoprotein; MI – myocardial infarction; _n – normal value; _a – abnormal value; TG – triglycerides; TC – total cholesterol; W – women; M – men. Data analyzed using the Kruskal-Wallis test.

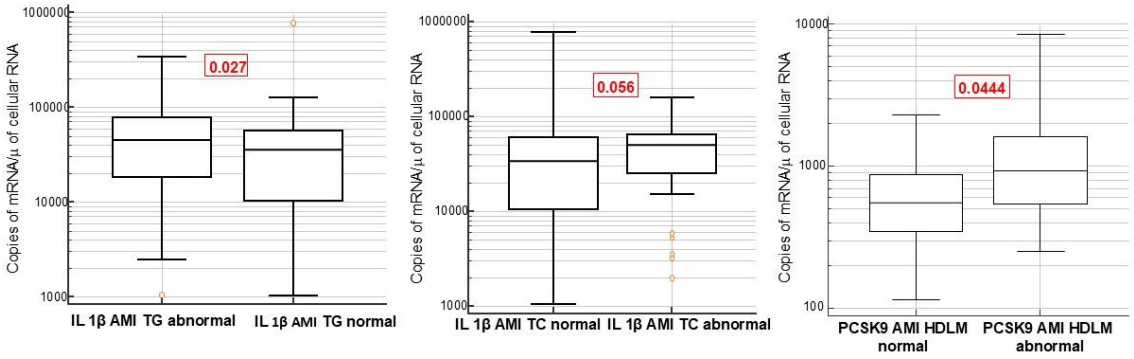

Fig. 2. Expression of interleukin 1 beta and subtilisin/kexin type 9 protein convertase genes in patients with acute myocardial infarction regarding lipid profile. IL-1β – interleukin 1 beta; AMI – acute myocardial infarction; TG – triglycerides; TC – total cholesterol; PCSK9 – subtilisin/kexin type 9 protein convertase; HDLM – high-density lipoproteins in men. Data analyzed using the Mann-Whitney U test.

DISCUSSION

PCSK9 plays a pivotal role in cholesterol metabolism by affecting the LDLR and reducing its levels on the plasma membrane [13]. In our study higher expression of PCSK9 was found in the group of patients with AMI compared to patients with CCS and those with excluded CAD ($p = 0.037$ and $p = 0.042$ respectively). This is consistent with the results obtained by other authors [14]. Available results of experimental studies indicate that inflammation can induce PCSK9 gene expression [15]. PCSK9 expression is not only restricted to the liver, but also occurs in the vascular wall and in inflammatory cells. The expression of PCSK9 was also demonstrated in atherosclerotic plaques [16]. Increase in PCSK9 concentration results in increased LDLR degradation, consequently leading to increased serum cholesterol levels. We have demonstrated increased expression of PCSK9 gene in case of atherogenic dyslipidemia. Additionally, it turned out that higher expression of PCSK9 gene occurred in the group of men and was associated with lower HDL concentration. This may be responsible for their worse prognosis. Further studies are needed to determine the influence of PCSK9 gene expression on lipid metabolism abnormalities and MI patient outcomes. PCSK9 expression may turn out to be valuable prognostic marker in that group of patients.

Atherosclerosis is considered to be an inflammatory disease. Atherosclerotic plaque formation is lipoprotein-dependent disease characterized by inflammation, proliferation, necrosis, and calcification in arterial wall [17]. Various inflammatory mediators and scavenger receptors have been implicated in inflammatory response in atherosclerosis. Plaque macrophages are able to modify, by oxidation, accumulated LDL to form ox-LDL [18]. LDLRs are not required for endocytosis of ox-LDL by monocytes/macrophages. Different scavenger receptors, like SRA, CD36, and LOX-1, are involved in this phenomenon. Their expression in highly increased by inflammatory stimuli [19]. There is evidence supporting direct relationship between PCSK9 and inflammation response. Ding et al. [20] demonstrated that in an inflammatory milieu, elevated levels of PCSK9 potently stimulate the expression of scavenger receptors and ox-LDL uptake in macrophages, and thus contribute to the process of atherogenesis. Human recombinant PCSK9 has been shown to directly activate macrophages as indicated by macrophage migration and release of proinflammatory cytokines [21]. There are also data confirming direct links between PCSK9 and IL-1β expression. Ding et al. [22] demonstrated that NLRP3 inflammasome induction via IL-1β induces PCSK9 secretion.

IL-1β is prototypical proinflammatory cytokine produced by macrophages, endothelial and smooth muscle cells. It is mainly activated by tumor necrosis factor alpha (TNF-α) as response to inflammation and plays multiple functions in blood vessel wall. Its atherosclerotic effect has been proven in experiments with the mouse model [23]. Due to its numerous and potent atherosclerotic effects on a number of cells, IL-1β appears to be one of the most important cytokines involved in inflammatory vascular diseases, especially atherosclerosis resulting in MI. IL-1β gene is located in chromosome 2. Gene expression is very complex process that depends on many factors. The pro IL-1β gene consists of seven exons with a primary transcription product length of 7008 nucleotides. IL-1β is formed as a precursor and is then activated in the inflammasome by caspase 1 with a significant contribution of NLRP3. It was shown that deletion of the IL-1 receptor was protective against ischemia and reperfusion injury in AMI models, resulting in smaller infarct size, reduced left ventricular enlargement and reduced left ventricular dysfunction [24]. It has also been shown that a single nucleotide polymorphism mutation in the IL-1β gene is associated with lower expression of basal C-reactive protein (CRP) concentration in healthy subjects. Elevated basal

CRP levels are, in turn, associated with increased cardiovascular risk [25]. Inflammasome and consequently production of active IL-1β form is stimulated by pathogen-related molecular patterns (PAMPs) and alarmins – damage-related molecular structures (DAMPs), which include cholesterol crystals. Ischemia and reperfusion related myocardial damage leads to impaired function of the viable myocardium, inducing an intense inflammatory response expressed by inflammasome activation, maturation of IL-1β and other proinflammatory cytokines [26]. Furthermore, IL-1β aggravates the dysfunction of myocardium resulting in its subacute damage, impaired contractility and a decrease in reactivity of beta adrenergic receptors. This leads to the stimulation of apoptosis, dilatation of the left ventricle and reduced response of beta adrenergic receptors [27]. There are many possibilities of pharmacological IL-1β blocking at different levels of its action.

We showed lower number of IL-1β gene transcripts in the group with AMI compared to the controls. However, majority studies conducted so far have shown an increased concentration of that cytokine in the post-infarction period [28,29]. These discrepancies can result from several issues. Firstly, in the majority of studies concentration of IL-1β and not, as in our study, gene expression, was assessed. Gene expression is the starting point for the formation of final product – protein. This is a multistage process. Even very high gene expression does not have to translate into a high concentration of the protein. Translation is controlled at many levels, starting with gene expression, its secretion and extracellular processing. In addition, it should be noticed that protein serum concentration applies to its entire pool, regardless of the source of its origin. In turn, gene expression (number of transcripts) refers to a specific population of cells – in this case, peripheral blood mononuclears, important for inflammation.

It is well known that the role of IL-1β is crucial in the postinfarction period, taking part in the inflammatory response, infarct healing and myocardial remodeling, which take place somewhat later [30]. In the AMI mouse model, it was shown that IL-1β genetic expression increases mostly in the first hours of acute ischemia, peaking at 6 hours since first symptoms, and then significantly decreases [31]. In our study blood samples for molecular testing were taken within the first 24 hours from hospital admission (not clinical presentation) which may explain lower expression of IL-1β in our study group. It seems that possible anti--inflammatory treatment in AMI should be ordered at the very beginning. This requires further clinical investigation.

In patients with AMI, increase in IL-1β gene expression was demonstrated in the groups with increased level of triglycerides ($p = 0.0564$) and total cholesterol ($p = 0.0444$). Available data regarding IL-1β gene expression and lipid profile in patients with AMI are scarce and often inconsistent.

Human atherosclerotic plaques are inflammatory lesions in which immune cells and inflammatory molecules are detectable in large levels. CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) confirmed the inflammatory theory of atherosclerosis and shed new light on the role of IL-1β in determining cardiovascular risk [32]. Canakinumab, an IL-1β blocking antibody, prevented the recurrence of ischemic events in patients who had experienced AMI in a study of 10,061 patients. Also phase II clinical trial showed promising data with anakinra, a recombinant IL-1 receptor antagonist, in patients with STEMI or heart failure with a reduced ejection fraction [33]. Unlike other anti-inflammatory drugs, in CANTOS, canakinumab did not affect cholesterol but slightly increased triglycerides, so that cardiovascular prophylactic effects were not dependent on any lipid effects related to IL-1β function.

Statins have become pillars of prevention and treatment of atherosclerotic cardiovascular disease. In addition to the lipid lowering effect, statins have various pleiotropic effects that include modulation of the inflammatory response [34]. Statins can increase PCSK9 expression thus in our study patients on statin therapy were not included into the study. Since the use of statins was an exclusion criterion, the recruitment process of patients and the control group was relatively long (about 2 years) – these were not patients subsequently hospitalized (all comers) due to MI, which is a certain limitation of this study. Abovementioned CANTOS study caused IL-1β has recently become an effective and relatively safe target for secondary cardiovascular prevention in patients with residual inflammation. Statins, although generally perceived as anti-inflammatory drugs, may have different effects on the synthesis of IL-1β in different cells, and some studies even show a paradoxical increase in its concentration after treatment with statins [35]. Given the deleterious role of IL-1β in the pathophysiology of MI, the addition of canakinumab to statins in these patients may provide a stronger inhibition of the IL-1β mediated inflammatory response.

It is commonly accepted that concentration of PCSK9 in AMI is increased, the data on the behavior of PCSK9 in the context of the advancement of CAD are inconclusive. We demonstrated that transcriptional activity of the PCSK9 in AMI patients increased along with advancement of CAD (number of significant coronary artery changes), but the difference turned out to be statistically insignificant. Interesting data in this regard was provided by Almontashiri et al. [14], which is an analysis of two independent, large, angiographic retrospective studies. Based on data analysis from

Ottawa Heart Genom Study (OHGS), it is known that in carefully selected 18 patients (without diabetes and without previous lipid-lowering treatment) with angiographically confirmed coronary heart disease, PCSK9 levels were not associated with the advancement of the disease. The same study found that PCSK9 levels were increased in the case of acute, but not previous, myocardial infarction. On the other hand, in the EmCB (The Emory Cardiology Biobank) study, in a similar group of patients, the concentration of PCSK9 was higher in the group of patients with angiographically proven coronary disease – the concentration of PCSK9 turned out to be an independent predictor of its occurrence. As in the OHGS group, also in the EmCB study, increased levels of PCSK9 were found in patients with acute, but not previously suffered, myocardial infarction.

Our study is not devoid of several limitations. In the field of study material, a significant limitation is the number of patients examined and overrepresentation of men in the studied group. The study is also limited by the single stage of genetic expression measurement – blood samples were taken within first 24 hours from admission. As mentioned above it could influence obtained results regarding especially IL-1β expression which can be time dependent in case of AMI. Finally, there is also lack of PSCK9 and IL-1β blood concentration assessed. However, this is the first step of research aimed to asses PSCK9 and IL-1β genetic expression in real-world AMI patients. Further exploring of PSCK9 and IL-1β expression and their relationship in cell models are planned.

CONCLUSIONS

Thorough understanding of IL-1β and PCSK9 biology, key representatives of two basic pathophysiological elements underlying MI, inflammation and lipid abnormalities, is of great practical importance. Known poor prognostic value of lipid disorders, such as increased concentration of total and reduced of HDL cholesterol, are reflected in the expression of PCSK9 and IL-1β. Further research is needed to assess the importance of predictive determination of IL-1β and PCSK9 genetic expression. This is particularly important due to currently wide availability of pharmacological intervention within metabolic pathways of these molecules.

Funding

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Medical University of Silesia, Katowice, Poland (approval No. KNW/0022/KBI/98/15).

Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

Study design – M. Majewski, J. Dąbek, Z. Gąsior
Data collection – M. Majewski, J. Głogowska-Ligus
Data interpretation – J. Dąbek, Z. Gąsior, A. Kułach
Statistical analysis – J. Głogowska-Ligus
Manuscript preparation – M. Majewski, A. Kułach
Literature research – M. Majewski, A. Kułach

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