PPARγ gene and atherosclerosis: genetic polymorphisms, epigenetics and therapeutic implications

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Abstract

Atherosclerosis is the leading cause of mortality and morbidity in the developed world. It is characterized by the formation of a plaque in the walls of middle and large arteries leading to macrovascular complications. Several risk factors are included, while diabetes is one of the most important for the onset and development of atherosclerosis. Due to an increase in the prevalence of diabetes in the world, the incidence of diabetic complications (microvascular and macrovascular) is increasing.

Peroxisome proliferator activated receptor gamma (PPARɣ) plays a important role in atherosclerotic processes. PPARɣ belongs to the superfamily of nuclear receptors, has a great presence in the fat tissue, macrophages and regulates gene expression and most of the processes that lead to the onset and development of atherosclerosis.

In this review, we discuss the basic pathophysiological mechanisms of atherosclerosis in type 2 diabetes mellitus (T2DM). Further, we discuss the impact of PPARɣ polymorphisms, and the epigenetic mechanisms affecting the onset of atherosclerosis, i.e. DNA methylation and demethylation, histone acetylation and deacetylation, and RNA based mechanisms.

Moreover, we add therapeutic possibilities for acting on epigenetic mechanisms in order to prevent the onset and progression of atherosclerosis.

Key note: Atherosclerosis; carotid atherosclerosis; coronary artery disease; PPARɣ polymorphisms; epigenetics of PPARɣ; therapeutic possibilities; pathophysiology

Introduction

Atherosclerosis is a long-term process characterized by plaque formation in middle and large arterial blood vessels [1]. Atherosclerosis is one of the leading causes of stroke, heart attack and peripheral arterial disease [2,3]. The prevalence and degree of atherosclerosis increases with increasing age, body mass index (BMI), increased blood pressure and serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) [4]. An elevated level of LDL is directly associated with development of atherosclerotic cardiovascular disease (ASCVD) [5]. According to the National Hearth, Lung and Blood Institute (NHLBI) data, atherosclerosis begins when certain factors such as: smoking, high cholesterol, high blood pressure and high blood sugar levels due to insulin resistance or diabetes damage the inner layers of the arteries. Other factors that have a significant effect on the development of atherosclerosis include family history, older age, unhealthy diet, lack of physical activity [2]. Depending on the position and size of the atherosclerotic plaque, microvascular and macrovascular complications of atherosclerosis can seriously damage the brain, heart, kidneys and other organs. Atherosclerotic disease of the carotid and coronary arteries appears to be highly prevalent in the ageing population [6].

Coronary atherosclerosis is the leading cause of coronary artery disease (CAD) [7]. Three pathological processes affect the formation of plaque: inflammatory reaction, cell proliferation and differentiation of foam cells [8]. The preliminary step in the formation of plaque is the passage of monocytes into subendothelial space and their differentiation into macrophages, which favor the oxidation of LDL particles in the blood and their endocytosis in the cells [9]. Endocytosis is mediated by scavenger receptors, which are not inhibited by content of cell cholesterol, that results in the accumulation of lipids in macrophages and foam cells. Activated macrophages secrete inflammatory cytokines (M-CSF, TNFa, and IL-1) that trigger inflammatory processes and lead to proliferation of smooth muscle cells. Then, macrophages

and foam cells necrotize, which leads to the release of the contents into the extracellular space, which is the basis for the onset of atherosclerosis [9]. PPARs modulate many aspects of these processes [10].

The aim of this review is to discuss the relationship between PPARɤ gene polymorphisms and macrovascular complications of carotid and coronary arteries in patients with type 2 diabetes (T2DM). Moreover, we discussed epigenetic mechanisms affecting the onset of atherosclerosis and therapeutic possibilities affecting epigenetic mechanisms in order to prevent the onset and progression of atherosclerosis.

PPARɤ and its role in the development of atherosclerosis

Peroxisome proliferator-activated receptors (PPARs) are members of the steroid/thyroid hormone receptor superfamily of transcription factors that are encoded by three *PPAR* genes: *PPARα*, *PPARβ/δ* and *PPARɤ*. *PPARɤ* has two isoforms of PPARɤ1 and PPARɤ2 which are ligand-activating transcription factors [9]. PPARɤ is the most prevalent in fatty tissue and macrophages and is very important in regulation of gene expression in metabolism and inflammation [11]. PPARɤ transcriptional activity was modulated by binding of numerous fatty acid metabolites that activate PPARɤ. Activated PPARɤ increases the expression of the scavenger receptor, which transmits ox-LDL from blood to macrophages, which then differentiates into foam cells [12]. By collecting foam cells, necrotic tissue residues, migrating and proliferating VSMCs (vascular smooth muscle cells), an atheromatous plaque is formed [13]. Overweights patients, Type 2 diabetics (T2DM) and non-diabetics, have increased PPARɤ (ɤ1 ,ɤ2) values, associated with changes in BMI and fasting insulin. Deviations from PPARɤ values indicate a possible role in the onset of insulin resistance of skeletal muscles in obesity and diabetes [9]. Thus, PPARɤ is involved in the regulation of all the steps that precede the

onset of atheromatous plaque, therefore the occurrence of mutations in PPARγ might be an initial step for the onset of atherosclerosis.

Polymorphisms of *PPARγ* gene - genetic biomarkers for atherosclerosis

Several studies have shown an association between the PPARγ polymorphisms and microvascular and macrovascular complications of coronary and carotid arteries in patients with T2DM [14–16].

In a study with a relatively small number of subjects (161) and a relatively young average lifespan (38 ± 15.3), Al-Shali and coworkers found a link between PPARγ genotypes and carotid atherosclerosis. They measured the thickness of carotid intima media (IMT) and total plaque volume (TPV), and found that subjects with *PPARG* A12 allele had lower IMT (0.72±0.03 mm; P=0.0045), without differences in TPV, and subjects with *PPARG* c.1431T allele have higher TPV (124±18.4; P=0.0079) without differences in IMT [14]. According to Li and coworkers, Pro12Ala polymorphism modulates PPARγ activity and leads to changes in the regulation of insulin sensitivity and glucose tolerance, which ultimately leads to CVD (allelic model: OR 0.80; 95%CI 0.66-0.98, P=0.040; dominant model: OR 0.74, 95%CI; 0.58-0.95, P=0.033). In their meta-analysis, 12 case-control studies (8 Caucasian, 3 Asian studies and 1 African study) were included with 10,189 cases with CVD (MI, CAD and ACS) and 17,899 control subjects [17]. Yan and coworkers found that the CC genotype of the C16T polymorphism (rs3856806) was associated with carotid lesions, while the CT + TT genotype had a protective role, indicating an important role of C161T polymorphism in carotid artery atherosclerosis. In the carotid artery of patients with metabolic syndrome, CC genotype vs. CT + TT genotype significantly increased IMT and Plaque index (IMT: 0.84 mm ±0.3 mm; plaque index: 2.19 ±1.21; P <0.05) [18]. In a study on Thai subjects, Yongsakulchai and coworkers found that the combination of *PPARγ* polymorphisms rs3856806 (C1431T), rs8192678

(G482S) and liver X receptor-α (LXRα) polymorphism rs12221497 (115G/A) predict the development and progression of coronary atherosclerosis in subjects at risk with CAD, and that the central role in this process belongs to rs8192678 polymorphism (OR 1.64, 95%CI: 1.01±2.66, P: 0.048) [13]. In their study, 387 subjects were included, aged between 35-85, of which 225 were with CAD and 162 non-CAD subjects (CAD group = stenosis ≥ 50% and non-CAD group = stenosis ≤50%, at least one of the major coronary arteries) [13].

Few studies have shown that there is no statistically significant relationship between PPARɤ polymorphisms and atherosclerosis [19–21]. Wang and co-workers in a meta-analysis involving 29 studies (15 Caucasian, 13 Asianstudies and 1 African study), with PPARɤ polymorphisms rs1801282 (Pro12Ala)/ rs3856806 (C161T) did not find a statistically significant relationship with the onset of atherosclerotic diseases [19]. In their meta-analysis, they analyzed different genetic models and associations with atherosclerotic disorders, there were no statistically significant results for the polymorphism rs1801282.

However, for the polymorphism rs3856806, based on ethnicity, they found a significantly increased risk for atherosclerotic disease in Caucasians (2679 cases with atherosclerotic disese and 5121 control subjects) for the additive model (OR 1.72; 95%CI 1.12-2.66) and for the recessive model (OR 1.71; 95%CI 1.11-2.62), whereas the risk for the Asian population (1910 cases with atherosclerotic disease and 1820 control subjects) has been reduced, for the dominant model (OR 0.70; 95%CI 0.62-0.81) and for the recessive model (OR 0.63; 95% CI 0.47-0.84).

Moreover, based on ethnicity, in the subgroup with myocardial infarction (MI): they reported an association for rs3856806 for the additive model (OR 2.68; 95%CI 1.10-6.54) and for the recessive model (OR 2.58; 95%CI 1.09-6.10), whereas for CAD they found decreased risk for the additive model (OR 0.67; 95%CI 0.51-0.88) and for the dominant model (OR 0.69; 95%CI 0.61-0.79) [19]. In the Korean Population Study, they did not find statistically significant

association between Pro12Ala polymorphism and CVD development (p=0.824). In their prospective study, 267 subjects were included, divided into four groups, in the number of stenotic coronary arteries, the values of stenosis ≥50% were considered significant. The percentage of patients with normal arterial lumen was 43.8%, 33% of patients had a stenosis of 1 coronary artery, 14.6% had a stenosis in 2 coronary arteries, and 8.6% had a stenosis of 3 coronary arteries [20]. Similarly, Wan and coworkers report that there is a significant link between the C161T genotype and the vessels disease in a group of Chinese patients with CAD and T2DM (OR 1.22; 95%CI: 1.03-1.45, P=0.019), but that there is no significant association with CAD risk (P = 0.695) [21]. In their study, a group of patients (CAD + T2DM) with CC genotype (70.3%) had severe stenosis >75% of one of the major coronary arteries. Moreover, they have discovered that the C161T polymorphism is associated with adipose metabolism, which suggests that by modulating it, the risk of atherogenesis can be reduced in the group of patients with CAD and T2DM [21].

Epigenetics

So far, no study has been reported about epigenetics of PPARγ in atherosclerosis in humans. However, epigenetic mechanisms have been implicated in the onset of atherosclerosis [22][23]. Previous research has shown a significant effect of epigenetic factors on gene expression, affecting adhesion, migration, differentiation of leukocytes, proliferation and migration of VSMCs, or all key processes in the onset and development of atherosclerosis. In addition to direct changes to human DNA, there are three other important epigenetic pathways that are important for the regulation of gene expression, which are DNA methylation, histone posttranslational modification and RNA-based mechanisms [22].

DNA methylation represents the covalent binding of the methyl group to the 5'position of the cytosine, and plays a very important role in the organization of chromatin and in that way leads

to the silencing of certain genes, the complex formed is called 5'methyl-cytosine [24]. Key role in DNA methylation, which is mainly related to the CpG region, is played by three methyl transferases (DNMT1, DNMT3a and DNMT3b) with the S-adenosyl methionine donor of the methyl group [24]. The exact mechanism of the development of atherosclerosis by changes in DNA methylation is not fully known, but many studies of high-fat diet-fed apoE null mice, human SMCs, and balloon-injured rabbit have shown a link between hypomethylation with the onset of atherosclerosis [25–27]. In these studies, hypomethylation was associated with increased expression of DNMT in atherosclerotic lesions, removal of the methyl group increases the transcription activity. Migration and proliferation of VSMC are the central axis in the development of atherosclerosis [26]. Lund G. and coworkers showed in a study with ApoE null mice that changes in DNA methylation profiles might be markers of atherosclerosis in diabetics [28].

The four classes of histones (H2A, H2B, H3 and H4) form an octameric complex, with two copies of each of these four histones, and 147 bp chromosomal DNA wrapped on it, forming the onset and functional unit of chromatin nucleosomes. Cell DNA is packaged in chromatin [29]. The unstructured N-terminal "tail" histone is subject to numerous modifications such as acetylation, methylation and phosphorylation [29]. The most common histone modification is acetylation.With the enzyme histone acetyltransferase (HATs) and histone deacetylase (HDACs), gene transcription activity is monitored, HATs add the acetyl group to the histone tail, thereby activating the gene, and HDACs inhibiting, removing the acetyl group [30]. Many studies show the relationship between acetylation and deacetylation status and atherosclerosis [26,31].

PPAR induces the expression of the nuclear receptor in macrophages (LXRα), which increases the expression of ABCA1 (a member of the ABC transporter protein family) leading to the elimination of cholesterol from the macrophage. Deacetylation of PPARɤ inhibits the pathway:

PPARɤ, LXRα, ABCA1, which leads to the blocking of cholesterol efflux, increased production of proinflammatory macrophages and the development of an inflammatory reaction, leading to the onset and development of atherosclerosis [32].

According to Cao and coworkers, the HDAC9 expression is associated with the onset of atherosclerotic plaques in the arteries, the onset of stroke, and the increased expression of macrophages acts atherogenetic [33]. In the study with LDLr - / - mice, the atherogenetic effect is reduced by deletion of HDCA9, which leads to an increase in macrophage cholesterol efflux and the prevention of the formation of foam cells, and reduces the production of inflammatory cells by translating macrophages from inflammatory M1 phenotype into an antiinflammatory M2 phenotype [33]. This study demonstrates the important role of HDCA9 in the development of atherosclerosis, and the possibility of developing epigenetic therapy aimed at inhibiting HDCA9 isoforms in macrophages.

The third epigenetic model, the RNA-based mechanism, is relatively new. Currently the greatest attention of scientists attracts non-coding RNA (ncRNA), including small RNAs [34]. Considering the length of the fragment, we distinguish two main types of ncRNA: long ncRNA (>200 nucleotides) and short ncRNA (<200 nucleotides) and several subtypes that modulate gene expression [34]. Short RNA (i.e. miRNA) performs genome repression by complementary binding to the 3'or 5' UTR region of targeted mRNA, activates the miRNA-induced silencing complex (miRISC) through which it silences gene expression [35]. LncRNA has a wide range of effects in various processes from increasing to reducing gene expression in combination with other epigenetic enzymes, plays an important role in chromatin modulation, transcriptional and post-transcriptional regulation, cell apoptosis, etc [35].

MiRNA has a leading role in the regulation of aterosclerotic process [36]. Previous studies of miRNA indicate a role in the prediction of certain diseases such as atherosclerosis, due to its

role in protein production and the impact of one miRNA on several hundred target genes, so far about 1100 miRNA is known in humans [36]. In the study, Zhao and coworkers point out the important role of miR613 in blocking the signaling pathway of PPARɤ, LXRa and ABCA1, which leads to the stopping of cholesterol efflux and the development of atherosclerosis. Also, indicating that activated PPARɤ increases the expression of LXRa and ABCA1, through the negative control of miR-613, acting anti-atherogenetic [37]. In the Tampere Vascular Study, a significantly expression of miR-21, miR-210, miR-34a, and miR-146a/b in aortic was reported in aortic, carotid, and femoral atherosclerotic arteries in relation to non-atherosclerotic left internal thoracic arteries [38]. MiRNA-21 and miRNA-34a show a significant relationship with the proliferation of VSMCs [39,40]. MiRNA-146a is associated with CADs and increased LDL release [40]. The levels of ox-LDL play an important role in the onset of atherosclerosis, increasing the expression of miRNA-29b. These effects are achieved by repression of DNMT3b, which increases cellular migration of VSMC through increased regulation of MMP-2 and MMP-9 [40]. In the study Cipollone and coworkers, a significant difference in the expression of miRNA-100, miRNA-127, miRNA-145, miRNA-133a, and miRNA-133b was found in the tissue of patients with endarterectomy of the carotid and control group [41]. In short, this study, as well as the above studies, points to the role of miRNA in the onset of atherosclerosis, and highlights the possibility of using miRNAs as biomarkers for the onset and development of atherosclerosis.

Therapy

In vitro and *in vivo* studies have shown a positive effect of TZDs (tiazolidinediones) on the function and pharmacology of β-cells through the mechanism of mediated PPARɤ, increasing the expression of PDX-1 (pancreatic duodenal homeobox) on β-cells in pre-diabetics and T2DM patients [42]. Clinical studies have shown that TZDs are PPARɤ agonists that reduce inflammatory reactions, modulate two ATP-binding cassette transporter (ABCA1 and ABCG1)

expression andinhibit key VSMC processes associated with atherosclerosis and protect blood vessels of T2DM patients [43]. Several studies have shown positive effects of TZDs (Troglitazone, Pioglitazone) in T2DM patients on intima media thickness reduction and restenosis processes in T2DM patients with stent [43–45]. On the other hand, several studies describe the opposite effect of TZDs on PPARɤ, which modulates adipocyte activity and leads to metabolic disorders and heart disease such as T2DM and CAD [17,46]. So far, delivery of miR-150 may represent a potential approach to prevent macrophage foam cell formation in atherosclerosis by inhibition of the formation of macrophage foam cells through targeting adiponectin receptor 2 [47]. Also, PPARgamma agonists, by activating PPARgamma, increase the concentration of adiponectin in plasma and expression of AdypoR2 in macrophages, they act anti-arteriogenic [48].

Conclusion

There are conflicting views on the role of PPARG polymorphism in the onset of atherosclerosis. The reason may be the different genetic background of the observed population. The current, meta-analyzes of the effects of PPAR polymorphism on the development of atherosclerosis are heterogeneous with an unclear conclusion. We believe it is important to do meta-analysis only in Caucasian or Asian populations, due to the impact of different genetic or epigenetic factors, which would contribute to a better understanding of the impact of these factors on the onset and the development of atherosclerosis in different populations. Some studies were done on a small number of subjects, some had a low average lifespan, therefore larger and prospective studies with homogeneous groups had to be done. It is also necessary to examine in more detail the effect of polymorphism investigated on the onset of atherosclerosis and their role in T2DM patients. Many studies have shown a significant effect of epigenetic factors in the development and onset of atherosclerosis but also other CVDs. However, the mechanism of origin is not adequately described, which must be accurately determined. Namely, epigenetic studies of

atherosclerosis can offer very good therapeutic solutions for CVDs and their prevention. Due to the contrary attitudes about the effect of TZDs therapy on the processes of atherosclerosis and the occurrence of adverse effects in T2DM patients with CVD, it is necessary to decisively investigate mechanisms of action of PPARγ agonists in order to prevent the onset and progression of atherosclerosis in T2DM patients.

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