REVIEW

Journal of Pre-Clinical and Clinical Research, 2009, Vol 3, No 2, 091-094
www.jpccr.eu

Atherosclerosis and mitochondrial dysfunction – possible links

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Abstract: Atherosclerosis is one of the most widespread diseases and remains a leading cause of death in developed countries. Traditional risk factors, such as obesity, cigarettes smoking, diabetes, and arterial hypertension, are insufficient for predicting which patients are in the group at highest risk for developing atherosclerosis. Knowledge about low-intensity inflammatory processes in the blood vessels, links between traditional risk factors and the forming of atherosclerotic plaques forming is constantly being extended, resulting in new strategies for treatment. Oxidative stress is described as an imbalance between reactive oxygen species (ROS) production and their elimination, in favour the former, and is believed to be characteristic of diabetes, hypertension and atherosclerosis. Increased level of ROS is considered an important cause of endothelial dysfunction occurring in the above-mentioned diseases by triggering the mitochondrial dysfunction. There is also evidence suggesting a possible link between mitochondrial dysfunction and cardiovascular diseases, thus indicating new directions for the examination of mechanisms of atherosclerosis.

Key words: atherosclerosis, oxidative stress, mitochondrial dysfunction, reactive oxygen species (ROS), reactive mitrogen species (RNS), endothelium dysfunction, 2-oxyglutarate

INTRODUCTION

Despite many preventive actions, atherosclerosis is still one of the most widespread diseases and remains a leading cause of death in developed countries. Knowledge about low-intensity inflammatory processes in the blood vessels, links between traditional risk factors, and the formation of atherosclerotic plaques forming is constantly being extended, resulting in strategies for new treatment. However, there are still many people who suffer from atherosclerosis complications, such as myocardial infarction, heart failure, brain stroke, peripheral arterial disease or renal failure – diseases which may significantly reduce physical ability and lower the quality of life.

Endothelium. The endothelium is the internal layer of blood vessels, and scientists formerly believed that it acted only as a passive barrier to blood elements. However, other endothelium functions, such as the regulation of vascular tone, supporting anticoagulative action and paracrinic activity have been discovered recently. Endothelial permeability plays an important role in the maintaining of albumin gradients, necessary for fluid balance in the tissues, and is enhanced by angiotensin, interleukin 1β or nitric oxide (NO). Vascular tone is regulated by endothelial secretion of vasodilator substances, such as NO and prostacyclines, and vasoconstrictor factors, e.g. angiotensin, endotelin-1, tromboxane A2, and superoxide anion.

The endothelium prevents the formation of thrombus by the synthesis and secretion of anticoagulative substances (antithrombin III, protein S, tissular factor inhibitor), platelets antiadherents (NO and prostacyclin), and fibrinolitic factors (plasminogen tissular factor), which is an advantage over the synthesis and secretion of procoagulative substances (von Willenbrand factor, fibronectin and thrombospondine). Another important role of endothelium is the synthesis and secretion of chemokines, activating the platelet factor, interleukin 8 (IL-8), and the chemotactic protein for monocytes (MCP-1), which enable lymphocytes and monocytes to migrate into the inner layer of arteries. Furthermore, vascular adhesion molecules, for example, vascular cellular adhesion molecule type 1 (VCAM-1), and intercellular adhesion molecule type 1 (ICAM-1), present on the surface of endothelial cells, also facilitate the transposition of leucocytes into the intima. In the presence of atherosclerosis risk factors, including cigarettes smoking, obesity, dyslipidemia, hypertension, and diabetes, the balance between different endothelium actions is disrupted. Such a status is called endothelium dysfunction and comprises the decrease of vasodilatating and anticoagulative endothelial actions and enhanced proinflammatory molecules synthesis [1, 2, 3].

Oxidative stress. Oxidative stress is described as an imbalance between reactive oxygen species (ROS) production and their elimination, in favour the former, and is characteristic of diabetes, hypertension and atherosclerosis. An increased level of ROS is considered an important cause of endothelial dysfunction occurring in these diseases [2]. ROS generated in blood vessels include superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hypochlorous acid, hydroxyl radicals ($^{\bullet}OH$) and singlet oxygen ($^1O_2$), with superoxide being the most importance [3]. ROS can directly inactivate endothelial-derived NO, cause protein dysfunction and cell signaling disturbances, which initiate and escalate endothelial dysfunction [2]. Many

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Received: 16 December 2009; accepted: 31 December 2009

enzymes, including NADPH oxidases, xanthine oxidaze (XO), myeloperoxidase (MPO), and nitric oxide synthases (NOS), are sources of ROS in vessels. However, the leakage of electrons from mitochondrial electron-transport chain is believed to be a major source of ROS [3]. Elevated inner mitochondrial membrane potential, calcium ions, and NO are expected to regulate electron-transport chain ROS production [4] . Complex III of the electron- transport chain is also believed to be a major source of ROS. On the other hand, the Krebs cycle enzymes, especially 2 -oxoglutarate dehydrogenase and pyruvate dehydrogenase, are also expected to generate ROS in the condition of increased NADH/NAD^+ ratio [3].

Reactive nitrogen species (RNS) generated in the vascular system, such as peroxynitrite ($ONOO^-$), a product of the reaction between superoxide and NO, are also considered as a cause of cell damage and death. Both ROS and RNS may cause oxidative modifications of low-density lipoproteins (LDL), thus enhancing the fomation of atherosclerotic plaques [1, 2, 3].

Antioxidants. There are several mechanisms of antioxidant actions. Intracellular antioxidants include superoxide dismutase (SOD) enzymes, which are metalloenzymes enabling the conversion of $O_2^{\bullet-}$ to H_2O_2. One of the SOD isoforms contains copper and zinc, and is located in the cytoplasm, another isoform is mitochondrial and contains manganese, while another SOD isoform is located in extracellular matrix, has been described. Reduced glutathione (GSH) peroxidase and catalase enable the conversion of H_2O_2 to H_2O in a condition that reduced glutathione (GSH) is available. GSH also acts as a direct antioxidant due to hydrogen ions donation [3].

According to present knowledge, ROS and RNS play an important regulatory role, which consists in the activation of mechanisms that control cell differentiation and the apoptosis processes [2, 3]. These processes are described as redox signaling and depend on posttranslational proteins modifications. It is known that ROS and RNS may also influence transcriptional factors activity. Among the transcriptional factors which remain under redox control are: nuclear factor- κβ (NF-κβ); hypoxia-induced factor 1 (HIF-1); nuclear factor (erythroid-derived 2)-like 2 (nrf2); and activator protein 1 (AP-1). In endothelial cells H_2O_2 improves the activity of NF κβ [5], which results in enhanced inflammatory response [2].

Mitochondrial damage. In conditions of oxidative stress, antioxidant defence systems are not sufficient to neutralize excessive amounts of ROS and RNS, which leads to protein, lipid, and DNA damage. Mitochondria are the most exposed to ROS and RNS organelles, and are also expected to be the most susceptible to damages caused by ROS and RNS [3, 6]. ROS and RNS are produced in mitochondria close to mitochondrial DNA, which is due to the lack of histons less prevented from oxidative damage than nuclear DNA. Also mitochondrial polymerases are more susceptible to caused by ROS modifications [3]. ROS may also induce cardiolipin oxidation due to it high content of unsaturated fatty acids and its close location to mitochondrial electron-transport chain [7], and due also to it decreasing complex I activity, leading to cytochrome c release [8].

According to recent data, mitochondrial dysfunction triggers cell necrosis and apoptosis pathways, thus playing a significant role in the development of diseases such as obesity, diabetes, heart failure, stroke, neurodegenerative diseases and cancer [3]. There is also evidence suggesting a possible link between mitochondrial dysfunction and cardiovascular diseases. In apolipoprotein E (apoE) knockout mice mitochondrial DNA oxidative damage was positively correlated with the extension of atherosclerotic plaques [9]. According to clinical data, patients with atherosclerosis, such as people with elevated cholesterol blood levels and smoking habit, revealed more disturbances in mitochondrial DNA in samples of their heart and arterial tissues than healthy controls [9, 10].

Oxidized lipids. Oxidized lipids may initiate cellular responses through either receptor-mediated or post-translational proteins modifications. It has been proved that low levels of oxidized lipids have a cytoprotective influence on cells, but high concentrations of oxidized lipids lead to cell apoptosis. Oxidized lipids are also expected to impair mitochondrial function [1, 3]. Endothelial cells incubation with oxidized LDL led to the induction of a mitochondrial complex I activity expected to depend on oxidative stress induction [11]. Recent data suggest that oxidized LDL may influence even more mitochondrial respiratory chain enzymes activity. Cultured porcine aortic endothelial cells incubation with oxidized LDL resulted in the decrease of NADH-ubiquinone dehydrogenase (complex I of mitochondrial respiratory chain), succinate cytochrome c oxidaze (complex II/III), ubiquinone cytochrome c reductase (complex III), cytochrome c oxidaze (complex IV), such as NAD^+/NADH ratio [12].

Another study revealed the induction the transcription and expression of mitochondrial SOD in human macrophages incubated with oxidized LDL [13]. It has also been proved that human macrophage incubation with oxidized LDL leads to enhanced production of mitochondrial ROS, and lowers mitochondrial membrane potential [14]. In another study, treatment of rat smooth muscle cells with oxidized LDL lowered intracellular ATP levels, such as mitochondrial oxidative phosphorylation subunits mRNA expression, impaired mitochondrial electron-transport chain capacity, insulin-mediated phosphorylation of Akt and AMP- activated protein kinase (AMPK). Mitochondrial dysfunction stimulated the migration abilities of these cells, probably through the inactivation of Akt, which may play a crucial role in the formation of atherosclerotic lesions [15]. However, increased production of ROS leads to vascular smooth muscle cells and macrophages apoptosis, thus resulting in the destabilisation of atherosclerotic plaques [16]. The destabilization of atherosclerotic lesions enables thrombus forming, which plays crucial role in the development of acute heart ischemia, which leads to myocardial infarction. Ischemia conditions trigger anaerobic metabolic pathways in heart tissue, which include the utilization of anaerobic glycolysis and fatty acids, enhanced glucose uptake, and decrease of heart muscle contractility.

In the case of persisting ischemia, the compensative mechanisms become insufficient and heart tissue develops significant ATP deficit; consequently resulting in increased calcium ions flow into the cell cytoplasm, xanthine oxidaze activation, and increased ROS generation leading to mitochondrial dysfunction [17, 18]. On the other hand, also reperfusion processes are involved with increased free radicals production, due to the leakage of electros from mitochondrial electron transport chain and xanthine oxidaze activation [17].

Mitochondrial dysfunction leads to increased ROS and RNS generation, which results in an enhanced intravascular inflammatory response, impaired endothelial NO synthesis, the dysfunction of the endothelium, and the initiation and progression of atherosclerosis. However, inflammatory cytokines, such tumour necrosis factor- α (TNF-α) enhance mitochondrial dysfunction through the increase of NAD(P)H oxidaze activity and ROS production [19]. Data suggest that also NO may play a regulative role in mitochondrial oxidative stress protection through an influence on peroxisome proliferators-activated

Mice with switched-off endothelial NO synthase activity revealed reduced levels of PGC-1α and reduced expression of genes involved in mitochondrial oxidative stress protection mechanisms, such as catalase, manganian SOD, peroxiredoxin III, peroxiredoxin V, thioredoxin 2 and thioredoxin reductase 2 [20]. Data also suggest that nitrite therapy after cardiac arrest protects against increased ROS generation during the reperfusion processes. In this experiment, nitrite-treated mice revealed a reversible inhibition of complex I of electron transport chain system resulting in decreased levels of reperfusion ROS production, but without any reduction in electron transport chain efficiency [21].

The Krebs cycle. According to recent data, the Krebs cycle inhibition seems to be an early marker of endothelium dysfunction. Chronic inhibition of NO synthesis in mice results not only, as expected, in elevated levels of endothelial dysfunction markers such as soluble ICAM-1, VCAM-1 and matrix metalloproteinase 9 levels, but also in the selective inhibition of Krebs cycle enzymes: aconitase-2 and enoyl-coA-hydratase-1, accompanied by reduced mitochondrial mass. These findings suggest that endothelial dysfunction results in Krebs cycle inhibition and enhanced glycolytic pathway of pyruvate usage, similarly to the hypoxia conditions [22]. 2-oxoglutarate dehydrogenase is another Krebs cycle enzyme which catalyses the conversion of 2-oxoglutarate to succinyl-CoA, thus producing NADH and providing electrons for electron-chain transport system, is expected to be also influenced by oxidative stress. As a result of increased ROS production, 2-oxoglutarate dehydrogenase inhibiting significantly lowers the supply of NADH to respiratory chain. On the other hand 2-oxoglutarate dehydrogenase also generates ROS by itself, when $NADH/NAD^+$ ratio is elevated [23].

2-oxoglutarate. 2-oxoglutarate (2-OG) is the Krebs cycle intermediate which is converted in the mitochondria into succinyl-CoA by 2-oxoglutarate dehydrogenase. Moreover, 2-OG is also contained in the cytoplasm and blood plasma where it is expected to act as a free ammonia scavenger, such as participating in proline synthesis in the intestine, and proline conversion to hydroxyproline [24, 25]. The origin of cytoplasmic and plasma 2-OG needs further explanation, but it has been proved that plasma 2-OG levels decrease together with the increase in the age of the patients [26].

According to recent data, oral 2-OG administration improved arterial wall elasticity and led to the increase of total collagen content in the walls of arteries in elderly mice [27]. Another study revealed that 2-OG treatment protects from oxidative stress cataract formation induced in rats. This suggests that 2-OG may act as ROS scavenger due to its alpha-keto-carboxylate group [28]. Another study proved the positive influences of 2OG on lipid peroxidation and antioxidant status in rats treated with ammonium acetate. In the study, rats treated with 2-OG were protected from developing metabolic disorders, such as the increase in free fatty acids, triglycerides, phospholipids, cholesterol, serum transaminases, and thiobarbituric acid reactive substances plasma levels, caused by ammonium acetate administration [29]. Data also suggest, that oral 2OG administration has beneficial influence on blood lipid levels. The study revealed that treatment with 2OG led to the decrease of total cholesterol, LDL and triglycerides in plasma, and the increase of HDL blood level in rats with experimentally-induced hyperlipidemia [30]. Recent data suggest that postnatal 2-OG administration has a protective influence on lipid metabolism in piglets prenatally exposed to dexamethasone. In the study, treatment with 2-OG resulted in a 40% reduction of total cholesterol plasma level, compared with the control group [31].

To summarise, atherosclerosis is one of the most widespread diseases in developed countries. Endothelium dysfunction is believed to play an essential role in the initiation and progression of formation of atherosclerotic lesions, and that oxidative stress is one of the most important risk factors that evokes and enhances endothelium function disorders, although knowledge about the links between traditional risk factors and the development of atherosclerosis continually comes to the fore. However, as long as it remains unpredictable which patients are in the group with the highest acute risk for coronary syndrome, and require the most aggressive treatment, and despite advanced treatment methods, it is still not possible to prevent some patients from developing atherosclerosis complications, further investigations are required.

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