

Hyperglycaemia and Inflammation are culprits of late diabetic complications

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Submitted: 28 May 2005

Accepted: 19 July 2005

Arch Med Sci 2005; 1, 2: 115-118

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Abstract

Hyperglycaemia and the inflammatory process play a crucial role in the development of late diabetic complications. In hyperglycaemic conditions glucose metabolism is enhanced in both main and alternative pathways. It results in enhanced glycation of proteins, glucose autoxidation and overproduction of reactive oxygen species (ROS). Metabolic disturbances lead to activation of protein kinase C (PKC) and nuclear factor kappa B (NF- κ B). NF- κ B is a transcription factor that itself activates many proinflammatory genes in the vasculature. Traditional risk factors such as elevated LDL cholesterol, low HDL cholesterol level, smoking, hypertension and hyperglycaemia do not fully explain the marked increase in mortality associated with diabetes and its complications. Many novel factors, named non-traditional cardiovascular disease risk factors, have been proposed recently as potentially important predictors of coronary heart disease both in general and diabetic population. Most of them are characteristic of the inflammatory process and reflect intensity of inflammation although experimental, clinical and epidemiological studies suggest that some processes related to low-grade inflammation may be relevant to diabetic micro- and macroangiopathy, further large-scale observational studies and randomized intervention studies of "anti-inflammatory" interventions will be needed to elucidate the meanings of these associations.

Key words: hyperglycaemia, inflammation, polymorphonuclear neutrophils, late diabetic complications.

Diabetic complications are the major cause of morbidity and mortality in patients with diabetes mellitus. Both acute and chronic hyperglycaemia are responsible for the development of micro- and macroangiopathy. The Diabetes Control and Complications Trial (DCCT) in Type 1 diabetes, and the United Kingdom Prospective Study (UKPDS) in Type 2 diabetes, have clearly shown that intensive control of hyperglycaemia can reduce the emergence and progression of retinopathy, nephropathy and neuropathy [1, 2].

Hyperglycaemia and inflammation

Inflammation is defined as a cascade of phenomena induced in response to different pathological stimuli. This physiological occurrence that allows restoring the homeostasis may also cause different diseases in the pathological conditions [3]. The inflammatory process seems to play an important role in the development of diabetes and its late complications [3, 4]. Both genetic and environmental factors, such as diet, physical activity,

smoking, stress are responsible for intensity of chronic low-grade inflammation. "Peaks and valleys" of glycaemia, insulin resistance and hyperinsulinaemia are independent accelerating factors mediating the tissue-injuring effects of inflammation [5-7].

There is a good evidence that hyperglycaemia induces biochemical and metabolic changes in tissues, mostly in cells that assimilate glucose independently to insulin. In hyperglycaemic conditions glucose metabolism is enhanced both by the main and alternative pathways [8]. It results in increased polyol pathway flux leading to accumulation of sorbitol, decreases the NADPH/NADP and increases the NADH/NAD⁺ ratio. The decline in cellular NADPH may decrease the generation of nitric oxide in endothelial cells and alter the cellular redox balance [9, 10]. The hyperglycaemia-induced increase NADH/NAD ratio is generally characteristic of hypoxia and called "metabolic pseudohypoxia" [11]. Hyperglycaemia enhances non-enzymatic glycosylation (glycation) of proteins and production of poorly reversible early glycation and completely irreversible Advanced Glycation End products. AGEs modify intra- and extracellular proteins. Moreover, through specific receptors for them (RAGE) it activates inflammatory cells such as monocytes, macrophages, endothelial and mesangial cells [12, 13]. The main alternative glucose metabolism pathway into the tissues with high expression of glucose transporters GLUT-4 (fat, muscle, heart) is hexosamine pathway. Upon entering cells, glucose is rapidly phosphorylated to glucose-6-phosphate, which can then be routed to the glycogen biosynthesis pathway or shunted to the pentose phosphate pathway. Alternatively, glucose-6-phosphate can be converted to fructose-6-phosphate and subsequently catabolized through the glycolytic pathway. These three pathways represent the major routes of glucose metabolism. However, glucose is also enzymatically converted to glucosamine-6-phosphate (GlcN-6-P) by glutamine: fructose-6-phosphate amidotransferase. GlcN-6-P undergoes rapid conversion to UDP-N-acetylglucosamine and CMP-N-acetylneuraminic acid, which are important precursor products in the biosynthesis of glycoproteins, glycolipids and proteoglycans. Formation of hexosamine products participates in molecular changes responsible for insulin resistance and transcriptional regulation of cellular functions [14].

These metabolic disturbances are dependent on glucose level and kind of cells and tissues. One of the tissues that are most sensitive to hyperglycaemia is endothelium. It was observed that hyperglycaemia enhanced glycolysis and mitochondrial overproduction of superoxide anions (O₂⁻) that are a very important substrate for other reactive oxygen species (ROS) [15]. Importantly, polymorphonuclear neutrophils (PMNs) are a huge source of ROS in the human body [16].

Biochemical disturbances such as hyperglycaemic pseudohypoxia, glucose auto-oxidation and increased production of AGE create specific constellation of different molecules with the central position of protein kinase C (PKC) and nuclear factor kappa B (NF-κB) activation [11, 12]. In endothelial cells, hyperglycaemia-induced PKC activation is associated with decreased bioavailability of nitric oxide and prostacyclin, increased synthesis of endothelin-1, and enhanced vascular permeability and prothrombotic state [15].

These factors affect the genes expression leading to molecular changes that were described by Brownlee as "hyperglycaemic memory" phenomenon [10]. Hyperglycaemic memory seems to be responsible for the development of late diabetic complications even of good metabolic control period, if hyperglycaemia has existed before.

Under hyperglycaemic conditions the activation of inflammatory and endothelial cells was observed. The physiologically friendly discussion between circulating leucocytes, platelets and endothelium changed into a heating argument leading to the damage of endothelium. Functional and structural changes of endothelium are involved in diabetic micro- and macronagiopathy.

Polymorphonuclear neutrophils and diabetes

The peripheral polymorphonuclear neutrophils are one of the main inflammatory cells and seem to significantly influence the damage of endothelium. Mechanisms of the injurious effect of activated neutrophils on endothelium are related to the release of large amounts of reactive oxygen species, proteolytic enzymes and cytokines. Additionally, increased neutrophil aggregation and adherence to endothelium were shown to result in leucoembolisation and capillary plugging with subsequent impairment of blood flow and tissue ischaemia. Physiologically PMNs die by programmed cell death named apoptosis (unstimulated cells) or by necrosis (activated cells) [17-19].

In diabetic patients, an increasing activity of peripheral circulating PMNs was noticed and simultaneously their worse response to stimuli [20, 21]. A number of circulating PMNs with expression of specific receptors CD11b/CD18, reflecting activation of cells, was significantly higher in diabetic patients compared with healthy subjects [22].

We have previously shown that the resting superoxide anions and hydrogen peroxide production by PMNs from patients with long-lasting Type 1 diabetes was elevated in comparison to control. Simultaneously, zymosan stimulated PMNs released significantly smaller amounts of reactive oxygen species. Furthermore, the diminished capacity of PMNs to generate superoxide anions in response to stimuli correlated with disease duration

[21]. Moreover, it was noticed that ROS production by PMNs in Type 2 diabetes was significantly associated with increment of postprandial glycaemia [23]. O_2^- and H_2O_2 production is rigidly connected with NADPH oxidase activation. It was clearly shown that the activity of this enzyme was increased markedly both in hyperglycaemic and hypoglycaemic conditions and reflects low grade inflammation [24].

Inflammation and late diabetic complications on the strength of Evidence Based Medicine

Traditional risk factors such as elevated LDL-C, low HDL-C, smoking, hypertension and hyperglycaemia are not fully explaining the marked increase in mortality associated with diabetes. Many novel factors, named non-traditional risk factors, have been proposed recently as potentially important predictors of CHD in the population. Most of them are characteristic of the inflammatory process and reflect intensity of inflammation [25-27]. It is not a surprise because there is good evidence that a low grade inflammatory process plays a crucial role in the development both atherosclerosis and diabetes and its complications.

Recently it has been shown that an easy and cheap-to-assess marker such as leucocyte count seems to be an independent risk factor for CVD as well as diabetic microangiopathy [28, 29]. Moreover, some studies indicate that neutrophil count may be a somewhat stronger predictor of CHD risk than other leucocyte components [30].

Prospective studies have demonstrated that the serum level of inflammatory markers is dependent on traditional risk factors of vascular diabetic complications such as duration of diabetes, smoking status, low physical activity and parameters of metabolic control (HbA1c, BMI, low HDL cholesterol, High triglycerides, hypertension) [7, 31].

There is good evidence that overeating, smoking, obesity and stress enhanced low grade inflammation via direct and indirect activation of NF- κ B [32]. Lack of physical activity affects not only metabolic abnormalities but also raises oxidative stress and inflammation. As expected, NAD(P)H oxidases overactivity is prevented by exercises [33].

High LDL cholesterol level induces oxidative stress and activates NF- κ B and fuels inflammation. On the other hand, high HDL cholesterol level has anti-inflammatory and antioxidant effect [34].

Hypertension directly and indirectly stimulates NAD(P)H oxidases leading to activation of NF- κ B and increased ROS production [35]. Thus, hypertension seems to have an inflammatory basis.

When we look at our patients with diabetes, we must think about their future and prognosis of late diabetic complications. If really hyperglycaemia and low grade inflammation are mainly responsible for the development of retinopathy, nephropathy,

neuropathy and macroangiopathy, we should give the following recommendations to diabetic patients at the onset of the disease: keeping stable normoglycaemia, pursuit of normal BMI, doing exercise and keeping fit, eating less and choosing healthy food, not smoking, being happy and, for people over 18, one glass of red wine would be advisable. All these recommendations have been demonstrated to decrease the low grade inflammation and increase the chance of having a long, sweet and happy life.

Although experimental, clinical and epidemiological studies suggest that some processes related to low-grade inflammation may be relevant to diabetic micro- and macroangiopathy, further large-scale observational studies and randomized intervention studies of "anti-inflammatory" interventions will be needed to elucidate the meanings of these associations.

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