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IMPLICATION OF PROINFLAMMATORY REACTIONS IN PATHOGENESIS OF DIABETIC MICROVASCULAR COMPLICATIONS

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Abstract Accumulating evidence supports that proinflammatory processes are implicated in the establishment of characteristic pathology in diabetic vascular and neurological complications. Altered cellular signaling during inflammatory processes is a new target for the treatment of diabetic complications and there appear to be some attempts to explore the effects of thiazolidinedione, a ligand of peroxisome proliferating activator receptor (PPAR)-γ for not only the treatment of diabetes itself but for the application to diabetic complications. We therefore explored the effects of PPAR-γ agonist, pioglitazone, on the experimental model of diabetic neuropathy. We found that pioglitazone significantly improved nerve conduction velocities and depressed protein kinase C activity. This is a new area for the exploration of effective treatment of diabetic complications that pose a considerable socioeconomic burden in the current world.

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Key words: inflammation; diabetes; vasculopathy; neuropathy; PPAR-γ

Introduction

Vascular and neurological complications pose a serious burden for diabetic patients because they lead to impaired quality of life and shortened life expectancy. Currently, we are shortage of effective treatment regimens for diabetic complications largely due to the fact that the pathogenesis of diabetic complications is still poorly understood. Nevertheless, continuous hyperglycemia is long considered to be a major culprit for the tissue injury specific to diabetes. Exposure to ambient high glucose exerts activation of polyol pathway, increased glycation of structural proteins and production of free radicals, thus leading to tissue damage[1]. Lowering of blood glucose or targeting of downstream molecules of hyperglycemic stimuli has been attempted to prevent or treat the development of diabetic complications, but yet to be satisfactory[2,3].

There are an increasing number of reports that development of diabetic complications is closely associated with proinflammatory processes[4,5]. Diabetic tissues prone to complications exhibit infiltrates of inflammatory cells, elevated synthesis and release of cytokines, enhanced signaling of intracellular protein synthesis, and cellular proliferation or death. It is therefore now to be explored to find a means that inhibits the proinflammatory processes for prevention and treatment of diabetic complications.

Inflammatory process in macroangiopathy

There is ample evidence that inflammatory process is implicated in the pathogenesis of macroangiopathy. Serum levels of C-reactive protein (CRP) are elevated in diabetic patients with thickened wall of carotid arteries, serving an index for atherosclerosis[6]. Recent epidemiologic survey disclosed an increase in CRP and the presence of carotid sclerosis even in prediabetic subjects with impaired glucose tolerance (IGT)[7]. During the course of

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atherogenesis, incorporation of oxidized lipids in macrophages forms fatty streaks in the intima, where migration and proliferation of medial smooth muscle cells contribute to typical sclerotic vascular wall. Within the sclerotic vascular wall, we frequently encounter infiltration of chronic inflammatory cells in the adventitia as well as media. These findings underlie the implication of proinflammatory processes in the vascular atherogenesis and the presence of diabetes accelerates such changes. The premise that inflammation is a causative factor for the atherosclerosis may be supported by the fact that chronic infection with EB or CMV virus infrequently accompanies atherosclerotic lesions in very young patients, in whom viral genome is demonstrated in the inflammatory cells in situ[8,9]. Likewise, patients with Helicobacter pylori infection, chlamydial infection or bacterial periodontal diseases commonly suffer from a high prevalence of atherosclerosis[10,11].

Inflammatory processes in microangiopathy

Very recently, inflammatory reactions are suggested to play a role in the cause and development of microvascular complications of diabetes. Serum inflammatory markers are elevated in patients with diabetic retinopathy[12] or nephropathy[13]. Experimental studies using diabetic animals also demonstrated a close association of proinflammatory activation in tissues that underwent changes analogous to human diabetic complications. In this setting, shortly after the initiation of diabetes, enhanced migration of macrophages and leukocytes was detected in retinal vessels, where inflammatory cells adhered to the surface of endothelial cells[14,15]. Increased infiltration of macrophages was noted in the kidney of diabetic mice[16] and mice deficient of macrophage scavenger receptor-a were shown to be resistant against diabetic nephropathy[17]. In the peripheral nerve obtained from diabetic rats, there occurs tissue damage by increased oxidative stress and migration of macrophages within endoneurium[18]. Not only in experimental diabetic animal models, epineurial vasculitis is also frequently detected in diabetic patients with proximal neuropathy or amyotrophy, inferring that proinflammatory activation or immune dysregulation may operate in the evolution of diabetic microvascular complications[19,20]. It appears therefore to be pertinent to explore the role of immune processes in the development of diabetic complications and as such effort may promote to find a novel approach for the treatment of diabetic complications.

Induction of inflammatory reactions by hyperglycemic stress

For the cause of diabetic complications, hyperglycemia in fact plays a major role in its pathogenesis. Under hyperglycemic conditions, enhanced polyol pathway hyperactivity, increased non-enzymatic glycation of structural proteins, advanced glycation end-products (AGE) formation and upregulation of receptor for AGE (RAGE) expressions, increased production of reactive oxygen species (ROS) and activation of poly-ADP-ribose (PARP) are all exerted for the tissue injury in a complicated manner (Figure 1).

Recently, Brownlee proposed a unifying hypothesis that mitochondria take a central position to produce unpaired electrons generating free radicals during increased oxidative phosphorylation of glycolysis, which secondarily enhances polyol pathway, AGE formation and an increase in protein kinase C (PKC) activity[21] (Figure 2).

Thus, the hyperglycemic stress causes oxidative stress to the vessels, which in turn causes an ischemic stress to the tissues. Consequent from hyperglycemic, oxidative and ischemic stresses, inflammatory reactions are elicited in injured tissues as a self-defense mechanism, recruiting inflammatory cells

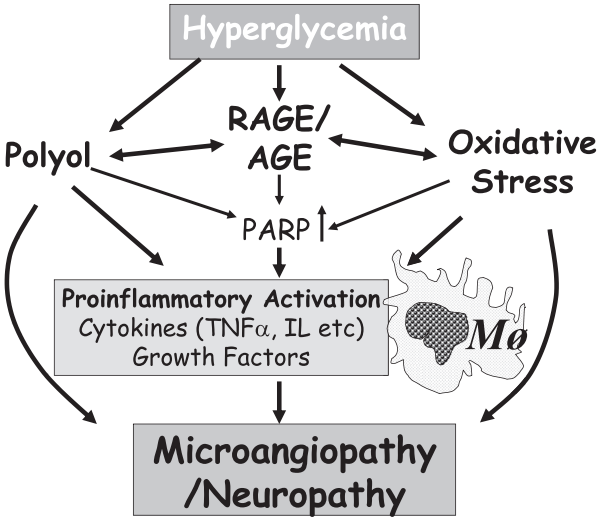

Figure 1 Schematic view of implication of inflammatory processes in the evolution of diabetic complications. Hyperglycemia stands central for the initiation of tissue injury eliciting several mechanisms like increased polyol pathway, enhanced protein glycation (AGE formation and active AGE-RAGE interaction) as well as increased oxidative stress. Then, in response to tissue injury, inflammatory processes are activated recruiting macrophage migration and release of cytokines. The inflammatory reactions augment the pathologic lesions.

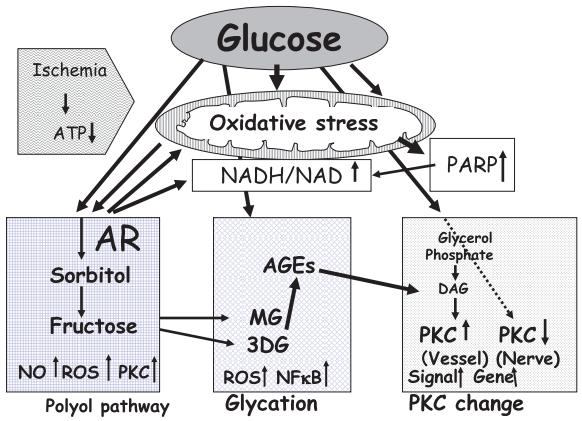

Figure 2 Unifying hypothesis for the cause of diabetic complications put forward by Brownlee (Reference #21). Hyperglycemia causes oxidative stress in mitochondria through oxidative phosphorylation of glycolysis. Then, all downstream pathways of polyol pathway, AGE/RAGE pathway and PKC activation are exerted. PKC activity in the vascular wall is raised while that in nerve tissues is decreased.

and release of inflammatory cytokines and chemoattractants. Thus, such inflammatory factors possibly contribute to the establishment of characteristic pathology of diabetic complications.

Signals that mediate inflammatory reactions and its clinical correlation

Under the circumstances of inflammatory activation, tissues prone to complications of diabetes undergo specific alterations in intracellular molecular signals for cell survival, proliferation or cell death with altered protein synthesis. By stimulation of cytokines or as a consequence of hyperglycemic, oxidative, and ischemic stresses, increased production of cell adhesion molecules, secretion of cell growth factors as well as increased stromal synthesis are all induced for the tissue remodeling. During this course, PKC activity is increased by de novo diacylglycerol synthesis with augmented expression of membrane PKC-β in the vessel walls[22], whereas PKC activity is decreased in nerve tissues due to reduced membrane expression of PKC-α[23,24,25]. As a downstream of elevated PKC activity, mitogen-activated protein (MAP) kinase is increased, thereby leading to activation of nuclear transcription factors of NF-κB as well as AP-1[22]. Such molecular alterations elicited by increased polyol pathway or excessive AGE deposition eventually cause either mitochondrial release of cytochrome C, caspase 3, and bcl-2, resulting not only in cellular dysfunction, but also to cell death[26,27].

In fact, there is an increased expression of aldose reductase[28], a key enzyme of polyol pathway, and deposition of AGE protein in human diabetic nerves[18]. Similarly, in experimental diabetic animal models, peripheral nerve tissues are accumulated with polyols and AGE, exerting proinflammatory processes via oxidative stress. In this condition, macrophages are excessively

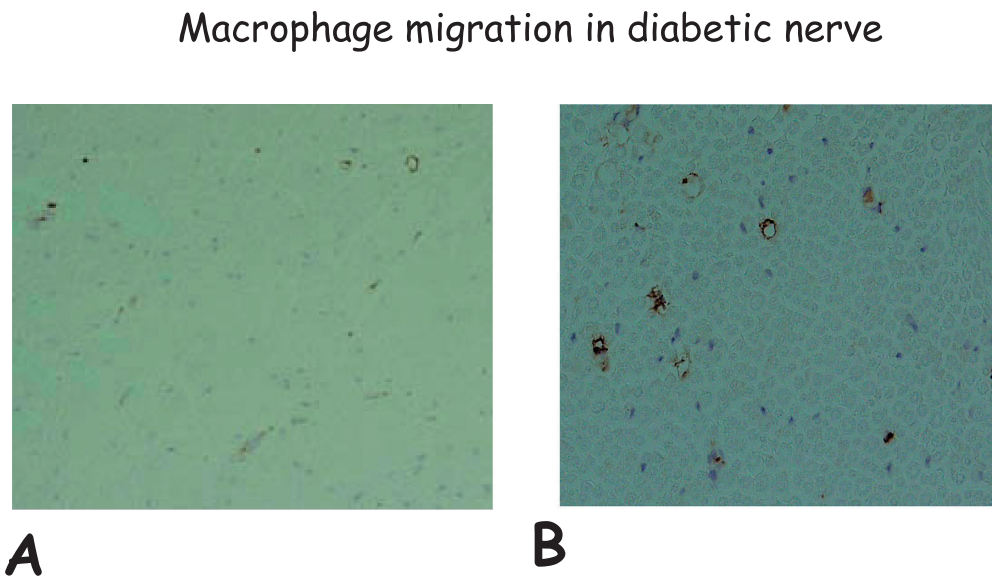

Figure 3 Migration of macrophages in the endoneurium of diabetic nerve (STZ-induced diabetic rat). Macrophages are identified by the positive staining with ED-1antibody. Compared with normal control rat (A), diabetic nerve contains more frequent appearance of ED-1 positive macrophages (B).

migrated within the endoneurium[18,29] (Figure 3).

Release of cytokines like TNF-α or interleukins from macrophages further augments tissue injury to establish the lesion. Recent studies demonstrated an activation of NF-κB and AP-1 by AGE-RAGE interaction that correlated with the complaint of pain in subjects with IGT[30] or conversely lack of pain in diabetic patients or diabetic animals[31], indicating that inflammatory processes may reflect the emergence of signs and symptoms of diabetic neuropathy. It is therefore expected that inhibition of proinflammatory processes could be applicable for controlling symptoms of diabetic patients and for the new treatment of diabetic neuropathy.

Anti-inflammatory agent and PPAR-γ ligand for diabetic complications

As alluded, it is a new trend to attempt to halt or inhibit the development of diabetic complications by treatment with anti-inflammatory agents. Most representative anti-inflammatory drug could be non-steroidal salicylate like aspirin and experimental studies confirmed the benefit of the use of these agents to prevent or inhibit the early vascular lesions in diabetes[32,33]. Based on the results from preclinical studies, it is now encouraged for these agents for the commencement of a long-term clinical trial on the efficacy on diabetic vascular complications[34]. Cox 2 inhibitors are also shown to ameliorate nephropathic and neuropathic changes in diabetic animals by suppressing inflammatory processes in tissues[35,36]. However, the long-term use of Cox 2 inhibitor is found to be problematic due to possible adverse effects.

Peroxisome proliferator- activator receptor (PPAR)-γ is a new nuclear transcription factor that mediates adipocyte differentiation and insulin action. Thiazolidinedione is a ligand to PPAR-γ and now widely used as an insulin sensitizer for type 2 diabetic patients[37,38]. It is now shown that PPAR-γ ligand possesses anti-inflammatory action mediated by inhibition of oxidative stress as well as macrophage suppression[37,39] (Figure 4).

The long-term clinical trial of pioglitazone,

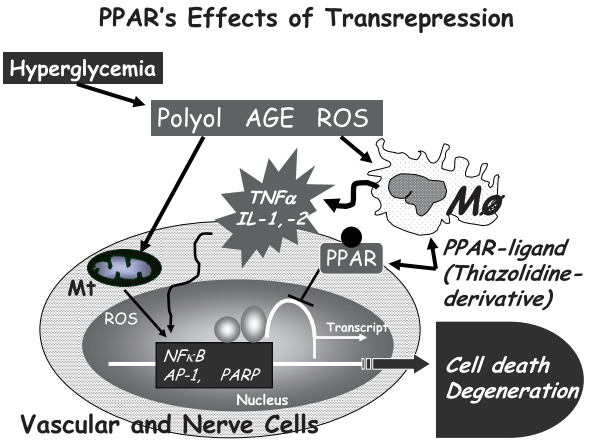

Figure 4 Hypothetical schema for the inhibition of inflammatory processes by PPAR-γ ligand thiazolididione. PPAR-γ ligand represses the process of activated transcription of NF-κB and AP-1 elicited by stimuli of TNFα or other cytokines. Simulatenously, PPAR-γ ligand also inhibits the macrophage activation.

representative compound of thiazolidinedione and PPAR-γ ligand, significantly inhibited the progression of macroangiopathy in diabetic patients[40]. There also emerge sporadic reports that demonstrated beneficial effects of thiazolidinedione on renal and neuropathic changes in experimental diabetic animal models[41,42]. However, mechanisms of how the compounds ameliorated tissue changes are largely unknown.

In our own studies, we attempted to use PPAR-γ ligand, pioglitazone, on experimental diabetic neuropathy using STZ diabetic rats. The severity of neuropathy was estimated by nerve conduction velocity (NCV), since it is known that this model suffers from severe NCV delay after the initiation of diabetes onset. To explore the mechanisms of how PPAR-γ ligand influenced the neuropathic processes, PKC activities and expressions of various isoforms of PKC were examined. The involvement of inflammatory processes was assessed by the extent of inflammatory cell migration within the endoneurium. As repeatedly reported, our model of STZ-induced diabetic rats also showed a significant delay of NCV, and pioglitazone treatment corrected the delay to almost normal levels without influence on blood glucose levels nor insulin deficiency. Biochemical analyses on sciatic nerve demonstrated suppression of PKC activity associated with decreased membrane expression of PKC-α isoform in untreated diabetic rats and again pioglitazone improved PKC activity and normalized the membrane expression of PKC-α protein. Cross sections of the nerve exhibited increased migration of macrophages in the endoneurium of diabetic rats and the number of macrophages was significantly less in pioglitazone-treated diabetic rats compared with those in untreated rats. The findings led us to conclude that pioglitazone was beneficial for the experimental diabetic neuropathy and may provide a rationale to test whether pioglitazone could be applicable for human diabetic neuropathy. It is interesting to see that diabetic patients treated with troglitazone, a prototype of thiazolidinedione, showed less frequency of neuropathic symptoms by epidemiologic survey[43]. It appears, therefore, that this field could be a new target for the treatment of diabetic complications although further analyses are apparently needed.

It is of note, however, that proinflammatory processes do not take a continuous phase, different from those found in sustained microorganism-caused infection. The processes found in diabetic microangiopathy appear to be the repetition of acute inflammatory processes and not typical of chronic inflammatory condition that is characterized by infiltration of lymphocytes and macrophages, as represented by the lesion in the vascular wall of macroangiopathy. It is therefore likely that the inflammatory processes elicited by hyperglycemia may be regulated in a tissue-specific manner that gives rise to tissue-specific pathology. This contention should be explored by further investigations.

Conclusion

Accumulating evidence suggests that inflammatory processes are strongly implicated in the pathogenesis of diabetic complications. This is not only related to hyperglycemia, but also to other metabolic abnormalities like hyperlipidemia and hypertension. Multidirectional approach should be essential for the better care for diabetic patients not to develop the miserable sequelae of complications in diabetes.

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