

PATHOPHYSIOLOGICAL FEATURES IN DIABETIC NEUROPATHY

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Abstract: Despite of the intense research of the last decades, there is no full understanding of the pathogenetical factors, their dynamics and how they correlate with structural and functional abnormalities seen in diabetic neuropathy (DN). DN results from damaging of neurons and axons by hyperglycemia and from neuronal ischemia which results from decrease of neurovascular flow. The vascular concept of DN involves the fact that endothelial dysfunction induced by diabetes with decrease of the blood flow in nerve and endoneurial hypoxia plays a key role in structural and functional abnormalities seen in diabetic nerve. Microangiopathy, or dysfunction of small blood vessels, is well related to the complications of diabetes, such as nephropathy and retinopathy, but its exact role in development of neural impairment is unknown. Defects in the vascular and metabolic ways interact with oxidative stress and produce the start and the progression of neural injury which is seen in DN. These ways include the formation of final products of advanced glycation, the alteration in ways of sorbitol, hexosamina and protein kinase C, and activation of poli-ADP ribose polimerase

Cuvinte cheie: neuropatia, microangiopatia, mitocondria, hiperglicemia, stresul oxidativ

Rezumat: În ciuda cercetării intense de-a lungul ultimelor decenii, nu sunt deocamdată pe deplin înțeleși factorii patogenetici, dinamica lor și cum se corelează ei cu anomaliile structurale și funcționale ce apar în neuropatia diabetică (ND). ND rezultă atât din afectarea hiperglicemică indusă per se a celulelor nervoase și a axonilor cât și din ischemia neuronală cauzată de scăderea fluxului neurovascular. Conceptul vascular al ND implică faptul că disfuncția endotelială indusă de diabet cu scăderea fluxului sanguin în nerv și hipoxia endoneurială joacă un rol cheie în modificările funcționale și morfologice din nervul diabetic. Microangiopatia, sau disfuncția vaselor mici, este strâns legată de complicațiile diabetului, cum ar fi nefropatia și retinopatia, dar rolul ei exact în dezvoltarea afecțiunii nervoase este incert. Defecte în căile metabolică și vasculară interacționează cu stresul oxidativ și produc debutul și progresia injuriei nervoase prezentă în ND. Aceste căi includ formarea de produși finali ai glicării avansate, alterarea în căile sorbitolului, hexozaminei și protein kinazei C și activarea polimerazei poli-ADP riboză

SCIENTIFIC ARTICLE OF BIBLIOGRAPHIC SYNTHESIS

Diabetic neuropathy (DN) is the most common late complication of diabetes mellitus with a prevalence varying from 10% within 1 year of diagnosis to 50% in patients with diabetes for more than 25 years (1,2,3)

Early metabolic and molecular changes in DPN has heavily relied on acute experiments in the streptozotocin (STZ)-diabetic rat. STZ-induced diabetes causes a partial β -cell destruction and hyperglycemia. It lacks the comorbidities characteristic of human type 2 diabetes such as obesity, hypercholesterolemia, and hyperlipidemia. The type 1 Bio-Breeding Worcester (BB/Wor)-rat develops acute onset of diabetes at the age of 70–75 days, secondary to an immune-mediated selective destruction of pancreatic β -cell. In the type 2 Bio-Breeding Zucker derived Worcester (BBZDR/Wor)-rat, outbred on the same background as the type 1 model, diabetes occurs at 70–80 days of age and is preceded by obesity. It develops peripheral insulin resistance with hyperinsulinemia, hypercholesterolemia and triglyceridemia and maintains spontaneously hyperglycemic levels equal to those of the type 1

BB/Wor-rat (4).

Both metabolic and ischemic mechanisms have a role in DN. Metabolic factors seem to prevail in length-dependent diabetic polyneuropathy (LDDP), whereas an inflammatory process superimposed on ischemic nerve lesions seems to be responsible for severe forms of focal neuropathies. The thickening and hyalinization of the walls of small blood vessels, which corresponds to reduplication of the basal lamina around endothelial cells, suggest a role for nerve ischemia in DN (5). Recent studies in patients with impaired glucose tolerance provide important insights into the role of the degree of glucose dysmetabolism in the development of neuropathy. The deleterious effect of hyperglycemia is confirmed by the occurrence of neuropathy associated with impaired glucose tolerance (6).

The potential role in DN of mitochondria of sensory neurons located in dorsal root ganglia has been suggested by several studies. These mitochondria are especially vulnerable, because in the hyperglycemic neuron they are the origin of production of reactive oxygen species, which can damage their DNA and membranes (7).

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Advanced glycation end products resulting from hyperglycemia act on specific receptors, inducing monocytes and endothelial cells to increase the production of cytokines and adhesion molecules (8).

Nerve trunks are supplied upstream by arterial branches of major limb vessels that share their supply with other limb tissues. In some nerve trunks, the centronervial portion of the nerve trunk might be the most vulnerable to ischemia. Ischemic damage of large multifascicular nerve trunks is more commonly multifocal, with irregular zones of axon damage that depend on specific features of their perfusion (9).

Spinal dorsal root ganglia are supplied from segmental radicular arteries and anastomoses with branches of spinal arteries. Peripheral nerve trunks are supplied by the epineurial vascular plexus and the intrinsic endoneurial blood supply. The epineurial plexus, is wellperfused by arterioles, has prominent arteriovenous shunting and has a leaky blood-nerve barrier. The endoneurium is largely supplied by capillaries that respond passively to changes in blood flow (10).

Some forms of focal diabetic nerve injury at "nonentrapment" sites might have an ischemic origin. For example, diabetic lumbosacral plexopathy is thought to be a consequence of focal plexus ischemia either from microangiopathy or superimposed vascular inflammation. Focal lesions or mononeuropathies are common and disabling in diabetes. Examples are carpal tunnel syndrome, intercostal neuropathies, and lumbosacral plexopathies. These focal peripheral nerve lesions regenerate more slowly in diabetics than nondiabetics and regeneration from them might be incapable of restoring function in many patients. Ischemic peripheral nerve lesions also have impaired regeneration (11,12).

Morphological studies of epineurial and endoneurial blood vessels in human sural nerve biopsies have identified microthrombosis and microvessel occlusion in diabetic nerves, endothelial duplication, smooth muscle proliferation, endoneurial capillary closure, basement membrane thickening, pericyte degeneration, and other changes. The loss of axons in a multifocal pattern in such biopsies has also suggested an ischemic or microvascular etiology (9,13,14).

The pathogenesis of DN involves hyperglycemia-initiated mechanisms as well as other factors, i.e., impaired insulin signaling, hypertension, disturbances of fatty acid and lipid metabolism. Two largest clinical trials in subjects with type 1 and type 2 diabetes, Diabetes Control And Complication Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS), indicate that intensive therapy and improved blood glucose control reduce incidence and slow progression of both complications, thus implicating hyperglycemia as a leading causative factor (15,16).

Mitochondrial oxidative phosphorylation is the major ATP synthetic pathway in eukaryotes. In this process, electrons from reducing substrates are transferred to molecular oxygen (O₂) via respiratory chain complexes I-IV. These complexes establish a hydrogen gradient across the inner mitochondrial membrane, and the electrochemical energy of this gradient is then used to drive ATP synthesis by ATP synthase (complex V). In the process of oxidative phosphorylation, energy carried by electrons is used by complexes I, III, and IV to pump protons out of the matrix. The resulting electrochemical gradient across the mitochondrial inner membrane is used by ATP synthase to drive the synthesis of ATP from ADP. In mitochondria, increased ATP synthesis is regulated by uncoupling proteins (UPC). Upon activation of these proteins, protons leak across the inner membrane and "uncouple" oxidative metabolism from ATP synthase, resulting in loss of ATP production. Basal and

hyperglycemia-induced ROS (reactive oxygen species) formation are decreased in dorsal root ganglia sensory neurons that over express UCP (17).

Under normal conditions, neurons have the capacity to neutralize both ROS and RNS (Reactive Nitrogen Species). Because O₂⁻ and H₂O₂ are normal products of the mitochondrial electron transport chain, SOD (superoxide dismutase), catalase, and glutathione are normally sufficient to remove these metabolic byproducts. Hyperglycemia increases mitochondrial activity and subsequent O₂⁻ production. Excess mitochondrial activity leads to an overwhelming production of ROS and RNS in a neuron that is already depleted of reducing equivalents and struggling with oxidative stress brought on by other metabolic and inflammatory insults. The buildup of ROS/RNS in the neuron coupled with the inability of the neuron to detoxify the excess ROS and RNS leads to progressive organelle, membrane and nuclear dysfunction (18).

Given the typical distal-proximal length dependent progression of diabetic neuropathy, axons are particularly susceptible to the metabolic and vascular imbalances that lead to diabetic neuropathy. Axons are susceptible to hyperglycemia not only because of their direct access to nerve blood supply, but also because of their large population of mitochondria. As these mitochondria become progressively dysfunctional, axons undergo energy failure which in turn precipitates axonal degeneration (18).

Advanced glycation endproducts (AGEs) are nonenzymatically created adducts between reducing sugars or oxaldehydes and proteins, DNA, or lipids. AGEs are thus heterogeneous, and are found both inside and outside the cell, where their formation interferes with multiple aspects of cell function. Extracellular formation of protein AGEs not only disrupt cellular adhesion (through interference with cell surface protein/extracellular matrix interactions), but also activate a specific cell-surface receptor for the AGEs, known as RAGE (19,20).

Activation of RAGE by extracellular AGEs leads to activation of the transcription factor nuclear factor kappa B (NF-κB), which regulates gene expression, apoptosis and inflammation. RAGE activation in diabetic animal models contributes to the onset and progression of diabetic neuropathy. RAGE activation in neurons also induces NADPH (Nicotinamide Adenine Dinucleotide Phosphate) oxidase activity, which further promotes mitochondrial oxidative stress and dysfunction (21).

The polyol pathway converts glucose to fructose through a two-step reduction/oxidation: First, aldose reductase reduces glucose to sorbitol, and then sorbitol dehydrogenase oxidizes sorbitol to fructose. Both aldose reductase and sorbitol dehydrogenase are prevalent in tissues prone to diabetic complications. The aldose reductase pathway is susceptible to over activation by a massaction effect of hyperglycemia, which results in imbalances of two of the pathway metabolites, NADPH and sorbitol. Excess glucose flow through the pathway causes consumption of NADPH, which is required for regeneration of reduced glutathione (22).

The depletion of glutathione secondary to excess aldose reductase activity thus renders the cell susceptible to oxidative stress. Increased production of sorbitol causes the intracellular environment to become hypertonic, and leads to compensatory efflux of other osmolytes such as myo-inositol (MI, important in signal transduction) and taurine (an antioxidant) (23,24).

Intracellular reducing potential is further diminished by the second step in the polyol pathway, the production of fructose. Hyperglycemia-driven production of excess fructose

promotes glycation and further depletion of NADPH. Activation of aldose reductase may also increase formation of diacylglycerol, which activates the deleterious protein kinase C pathway. Patients with a "high aldose reductase expression" genotype are commonly found to have early DN while patients with a "low aldose reductase expression" genotype are less susceptible to neuropathy (25,26,27).

Excess available glucose causes a mass action increase in flux through the hexosamine pathway. Under normal circumstances, a small amount of the glycolytic intermediate fructose-6 phosphate is shunted from glycolysis to the hexosamine pathway. The hexosamine pathway converts fructose-6 phosphate to glucosamine-6 phosphate by glutamine fructose-6 phosphate amidotransferase. Glucosamine-6 phosphate is then converted to uridine diphosphate- N-acetyl glucosamine (UDP-GlcNAc), which is the obligatory substrate for O-GlcNAc transferase, attaching O-GlcNAc to the serine and threonine residues of transcription factors and altering gene expression (28).

Impaired fibrinolysis in small neural blood vessels promotes nerve ischemia, leading to oxidative stress and the signs and symptoms of DN. Plasminogen activator expression is lower by four to six fold in the epineurial and endoneurial microvessels in sural nerves from patients with DN compared to control nerve biopsies. This lower expression would promote thrombosis and nerve ischemia. Patients with DN had higher serum levels of plasminogen activator inhibitor-1 (PAI-1) complexes than those men without neuropathy (29).

Hyperglycemia stimulates over-activation of the protein kinase C (PKC) pathway by increasing synthesis of diacylglycerol (DAG), which activates PKC. The PKC β -isoform in particular has been linked to the development of retinopathy, nephropathy, and cardiovascular disease. Hyperstimulation of PKC causes the overexpression of the angiogenic protein vascular endothelial growth factor (VEGF), PAI-1, NF- κ B, and TGF- β , supporting a role for PKC activation in the pathogenesis of DN. PKC-induced vasoconstriction, altered capillary permeability, hypoxia, and nerve basement membrane thickening are all thought to be involved in DN (30,31)

Poly-ADP ribose polymerase (PARP), a nuclear enzyme closely associated with oxidativenitrosative stress, is expressed in sensory neurons, Schwann cells, and endothelial cells. While hyperglycemia, free radicals, and oxidants stimulate PARP activation, PARP also causes oxidative stress. PARP cleaves nicotinamide adenine dinucleotide (NAD⁺) to nicotinamide, and also removes ADP-ribose residues attached to nuclear proteins (32).

Elevated blood levels of inflammatory proteins, including C-reactive protein and TNF- α , are associated with neuropathy. Hsp 27, part of the TNF- α signaling pathway that leads to release of the inflammatory mediators cyclooxygenase-2 (Cox-2), IL-6, and IL-8, was recently found by the Eurodiab study to be elevated in the blood of diabetic patients with neuropathy (33,34).

Excess glucose-mediated activity in the hexokinase and PKC pathways results in activation of signaling intermediates and modified transcription factors, ultimately increasing TGF- β and NF- κ B (28).

RAGE activation by extracellular AGEs also affect inflammation by causing the upregulation of NF- κ B, which in turn upregulates Cox-2. Cox-2 stimulates production of prostaglandin E2 and ROS, which go on to further activate NF- κ B. NF- κ B/Cox-2 upregulation is present in the vasculature and peripheral nerves of animal models of diabetes (35,36).

NF- κ B participates in a second vicious cycle of

inflammation, in which it both induces and is induced by inducible nitric oxide synthase (iNOS). NO produced by the excess of iNOS contributes to microvascular damage by diminishing the blood supply to nerve (37).

NF- κ B appears to be the keystone of the inflammatory pathways that participate in the development of diabetic neuropathy. Chronic NF- κ B activation appears to render neurons and blood vessels more susceptible to ischemia-reperfusion injury. The subsequent extensive infiltration of macrophages is further intensified by NF- κ B-stimulated release of cytokines from endothelial cells, Schwann cells and neurons. The activation of macrophages leads to further production of cytokines, as well as proteases and ROS that lead to myelin breakdown, cellular oxidative damage, and impairment of nerve regeneration (38,39).

In conclusion, multiple mechanisms are involved in the pathogenesis of DN. New findings support the role for previously discovered mechanisms, such as increased AR activity, nonenzymatic glycation, PKC activation, and oxidative stress in functional and morphological abnormalities in the diabetic nerve. Several newly discovered mechanisms include activations of NF- κ B, the 12/15-LO (12/15 lipoxygenase) pathway, and NHE-1 (Na/H exchanger). Studies of the role for these mechanisms in DN and their interactions with other pathogenetic factors are in progress.

Microangiopathy involving vessels of the nerve trunk and those of dorsal root ganglia does develop in parallel with neuropathy and is likely to eventually contribute to it. Failed upregulation of blood flow to injured nerves after acute injury might impair their ability to regenerate. It is probably incorrect to conclude that microvascular disease is the primary trigger of neuropathic complications, an assumption that ignores direct neuronal damage. It might be more accurate to depict chronic diabetes as involving nerve trunks, ganglion, and their respective microvessels in parallel, a process that can eventually lead to a vicious interactin cycle of damage.

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