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European Journal of Pharmacology 375 (1999) 217–223

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Review

Glucose-induced oxidative stress and programmed cell death in diabetic neuropathy

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Accepted 30 April 1999

Abstract

The Diabetes Control and Complications Trial (DCCT) established the importance of hyperglyemia and other consequences of insulin deficiency in the pathogenesis of diabetic neuropathy, but the precise mechanisms by which metabolic alterations produce peripheral nerve fiber damage and loss remain unclear. Emerging data from human and animal studies suggest that glucose-derived oxidative stress may play a central role, linking together many of the other currently invoked pathogenetic mechanisms such as the aldose reductase and glycation pathways, vascular dysfunction, and impaired neurotrophic support. These relationships suggest combinations of pharmacological interventions that may synergistically protect the peripheral nervous system (PNS) against the metabolic derangements of diabetes mellitus.

Keywords: Glucose; Diabetic neuropathy; Programmed cell death

1. Introduction

Diabetic neuropathy is the commonest peripheral neuropathy in developed nations, affects most patients with diabetes (Dyck et al., 1993), and is the leading cause of non-traumatic lower limb amputations in diabetic patients. In the US, diabetic neuropathy accounts for ~60,000 amputations/year. Although diabetic peripheral neuropathy is a multifactorial disorder, it is conditioned by hyperglycemia and/or insulin deficiency (The DCCT Research Group, 1993, 1995a,b), and is characterized by a complex pathogenetic network of interrelated metabolic, neurotrophic and vascular defects (Dyck, 1989; Tomlinson et al., 1994, 1996; Stevens et al., 1995; Cameron and Cotter, 1997; Garrett et al., 1997). Together, these initiate chronic progressive damage and loss of unmyelinated and myelinated nerve fibers, that eventually culminate in a distal symmetric sensorimotor peripheral polyneuropathy. The interrelationships and cellular localization of the metabolic consequences of hyperglyemia that initiate the pathogenetic cascade remain highly speculative (Stevens et al., 1995; Tomlinson et al., 1996; Cameron and Cotter, 1997), and are confounded by the complex, composite, and multicellular nature of peripheral nerve, composed of myelinated and unmyelinated axons, their associated Schwann cells, endoneurial macrophages, the perineurial membrane, and endoneurial and perineurial microvessels, epineurial arteries and arterioles. Distant dorsal root sensory neurons and spinal motor neurons that contribute peripheral nerve axons are an integral part of this multicellular structure, as are the distant sensory and motor end-organs.

2. Therapies with established ''proof of concept''

Besides improved blood glucose control (The DCCT Research Group, 1995a,b), there is no generally accepted clinically proven treatment to prevent or ameliorate the nerve damage responsible for diabetic peripheral neuropathy. Several promising therapies have undergone successful ''proof-of-concept'' studies in patients with diabetic neuropathy. Aldose reductase inhibitors improve nerve

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00356-8

conduction velocity and nerve morphology in patients with diabetic peripheral neuropathy (Greene et al., in press). Therapy with nerve growth factor (NGF) improves unmyelinated fiber morphology and function in diabetic mice (Elias et al., 1998) and improves sensation and sensory function in patients with diabetic neuropathy (Apfel et al., 1998). Short-term administration of the antioxidant DL-α-lipoic acid has been reported to improve painful symptoms (Ziegler et al., 1995) in human diabetic peripheral neuropathy. The specific pathogenic components individually targeted by these promising therapies have not been rigorously studied at the biochemical, molecular or cellular level, where the potential for significant overlap and/or synergy may exist. Knowledge of this synergy may have important implications in the design and testing of comprehensive therapeutic regimens for diabetic peripheral neuropathy.

3. Oxidative stress: a possible locus of therapeutic synergy

Recently, ''oxidative stress'', defined as the generation of toxic reactive oxygen species (Sies, 1997), has been invoked as a possibly critical factor in the development of diabetic peripheral neuropathy (Cameron et al., 1993a,b, 1994; Karasu et al., 1995; Matsumoto et al., 1995; Nagamatsu et al., 1995; Van Dam et al., 1995; Cameron and Cotter, 1996; Nickander et al., 1996; Low et al., 1997a,b; Stevens et al., submitted). Oxidative stress may constitute a focal point for multiple therapeutic interventions, and for therapeutic synergy. Hyperglycemia is thought to promote oxidative stress through both non-enzymatic and enzymatic mechanisms. Non-enzymatic protein glycation is thought to generate reactive oxygen species through a complex series of chemical and cellular intermediates (Lai et al., 1993, 1995). Reduction of glucose to sorbitol by aldose reductase also is thought to promote oxidative stress (Nagamatsu et al., 1995) by shifting the nicotinamide dinucleotide phosphate ($NADP^+$/NADPH) and glutathione (GSH/GSSG) redox couples towards the more oxidized forms (Van Dam et al., 1995). Moreover, accumulation of sorbitol produces reciprocal depletion of taurine, an intracellular osmolyte and endogenous antioxidant (Aruoma et al., 1988), compromising antioxidative defense (Cameron and Cotter, 1993). Thus the aldose reductase pathway may contribute to oxidative stress through both co-factor and osmotic mechanisms. Accumulation of conjugated dienes (Low and Nickander, 1991), and reductions in superoxide dismutase (Low and Nickander, 1991), glutathione peroxidase (Hermenegildo et al., 1993), and taurine (Stevens et al., 1993) are regarded as evidence for increased reactive oxygen species damage and reduced oxidative defense in experimental diabetic peripheral neuropathy.

Recent biochemical and pharmacological studies of experimental diabetic peripheral neuropathy in the streptozotocin-induced diabetic (STZ-induced diabetic) rat model (Stevens et al., submitted) support the proposition that oxidative stress may bridge across many glucose-related pathogenetic pathways. These studies attribute reduced nerve blood flow (Cameron and Cotter, 1993, 1995a,b, 1996; Cotter and Cameron, 1995; Karasu et al., 1995; Nagamatsu et al., 1995; Low et al., 1997a,b) in experimental diabetic peripheral neuropathy to reactive oxygen species-induced vascular damage (Cotter and Cameron, 1995; Cameron and Cotter, 1997; Stevens et al., submitted). Conversely, endoneurial ischemia may accelerate reactive oxygen species-related damage to the cellular elements of peripheral nerve tissue (Low et al., 1997a,b). Vascular impairment may reduce nerve conduction velocity directly by reducing ATP needed for membrane repolarizaton (Tuck et al., 1984; Stevens et al., submitted), or indirectly by inducing oxidative stress (Low et al., 1997a,b). Sensory nerve fibers may be particularly sensitive to oxidative stress (Nagamatsu et al., 1995; Stevens et al., submitted). Depletion of antioxidants such as GSH (Nagamatsu et al., 1995) and taurine (Stevens et al., 1993), consequent to aldose reductase pathway activation, may enhance protein kinase C (PKC) activation (Li and Lombardini, 1991; Ward et al., 1998), further contributing to vascular dysfunction (Koya and King, 1998) and nerve ischemia. Both reactive oxygen species (Li et al., 1996) and glucose-induced *myo*-inositol depletion (Shindo et al., 1996) may together impair neuronal phosphoinositide signaling. This relationship is supported by the recent observations that antioxidant therapy ameliorates nerve *myo*-inositol depletion (Low et al., 1997a,b; Stevens et al., submitted). Oxidative stress may diminish NGF-mediated neurotrophic support (Garrett et al., 1997), and NGF upregulates neuronal antioxidative defense enzymes (Jackson et al., 1990a,b; Nistico et al., 1992). Thus, oxidative stress is closely intertwined with most of the proposed pathogenic cascades for diabetic peripheral neuropathy involving nerve ischemia, PKC, aldose reductase, osmolytes, glycation and neurotrophism.

4. Diversity of primary glucotoxic mechanisms in diabetic nerve

Animal and in vitro experiments implicate both enzymatic and non-enzymatic metabolic mechanisms in the initiation of glucose-induced neurotoxicity. Implicated metabolic initiators include non-enzymatic glycation of proteins with subsequent chemical rearrangements yielding complex protein adducts known as ''advanced glycation end-products'' (Schmidt et al., 1994; Varma et al., 1997). Glucose may undergo non-enzymatic auto-oxidation (Love et al., 1996). Increased aldose reductase pathway activity

may lead to accumulation of sorbitol and fructose, NADP-redox imbalances as well as alterations in signal transduction (Greene et al., 1993; Stevens et al., 1994; Cameron and Cotter, 1997), and activation of PKC perhaps due to increased de novo synthesis of diacylglycerol from glucose and inhibition of diacylglycerol kinase (Inoguchi et al., 1992; Bursell et al., 1997; Cameron and Cotter, 1997; Ishii et al., 1998). These metabolic initiators are compartmentalized within the rich anatomical complexity and cellular heterogeneity of the peripheral nervous system (PNS) and its supporting vasculature and connective tissue elements. This compartmentalization channels and shapes the physiological consequences of the metabolic initiators into the specific nerve fiber damage and loss that underlies diabetic peripheral neuropathy. The intervening physiological mediators include interruption of nerve blood flow (Cameron et al., 1994; Stevens et al., 1994; Cotter et al., 1995; Nagamatsu et al., 1995), mitochondrial dysfunction (Stevens et al., 1994; Obrosova et al., 1997), impaired neurotrophic support (Fernyhough et al., 1998), osmolyte derangements (Cameron et al., 1997a,b), and induction of neuronal and/or Schwann cell apoptosis (Ekstrom, 1995). Combinations and permutations of metabolic initiators, cellular and subcellular compartmentalization, and physiological mediators give rise to the current spectrum of pathogenetic hypotheses for diabetic peripheral neuropathy.

5. The biochemical basis of glucose-induced oxidative stress

Auto-oxidation of glucose, catalyzed by trace amounts of free transition metals such as iron and copper (Wolf, 1993), generate reactive oxygen species in vitro (Jiang et al., 1990). This process is implicated in the pathogenesis of experimental diabetic peripheral neuropathy by the ability of metal chelating agents to preserve normal nerve conduction velocity and nerve blood flow in diabetic rats (Cotter and Cameron, 1995) (transition metal handling may be impaired in experimental diabetes) (Cutler, 1989) (Fig. 1, yellow). Auto-oxidation is closely interconnected with advanced glycation end-product formation: reactive oxygen species accelerate advanced glycation end-products formation and advanced glycation end-products in turn supply reactive oxygen species (‘‘auto-oxidative glycosylation’’; Baynes, 1991). Advanced glycation end-product formation is thought to produce reactive oxygen species (Fig. 1, yellow) through a series of complex biochemical and molecular pathways (Mullarkey et al., 1990; Van et al., 1994; Giardino et al., 1998). Binding of advanced glyca-

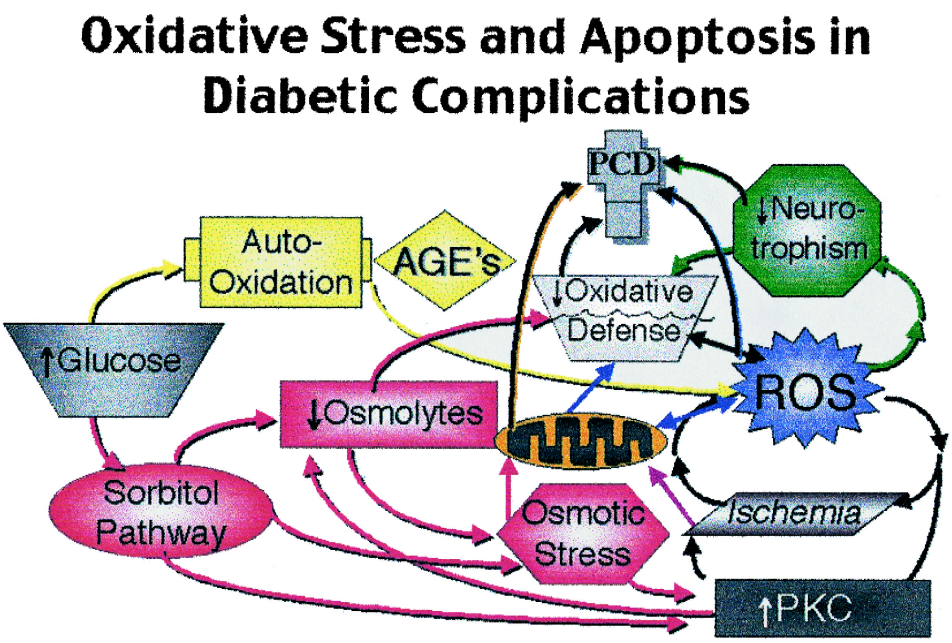

Fig. 1. Increased glucose is thought to initiate a cascade of putatively cytotoxic metabolic events through autoxidation, glycation and formation of advanced glycation endproducts (AGEs) (yellow), and through increased sorbitol pathway activity (red). Autoxidation and/or AGEs are thought to promote the generation of toxic free radicals, including a variety of reactive oxygen species (ROS) (blue). Sorbitol production produces compensatory depletion of other organic osmolytes (red) such as *myo*-inositol and taurine, an endogenous antioxidant, with resulting attenuation of oxidative defense (dark gray). Sorbitol accumulation and/or reciprocal osmolyte depletion also produce osmotic stress (red), which may damage mitochondria and stimulate PKC. PKC may also be activated by shifts in triose phosphates towards diacyglycerol production as a result of increased sorbitol pathway activity. Both ROS and PKC activation have been implicated in microvascular dysfunction in peripheral nerve and retina, producing tissue ischemia (purple). Ischemia impairs mitochondrial function and generates additional ROS. Mitochondrial dysfunction, and ROS further impair oxidative defense mechanisms. Neurotrophic support through NGF and perhaps other neurotrophins (green) that regulates oxidative defense mechanisms, may be impaired by ROS. Mitochondrial damage is thought to lead to the release of cytochromes, activation of caspaces, and induction of apoptosis or programmed cell death (PCD).

tion end-products to their cell surface receptor is associated with activation and nuclear translocation of the transcription factor NFkB (Bierhaus et al., 1997), possibly contributing to endothelial dysfunction (Amore et al., 1997; Pieper and Riaz-ul-Haq, 1997) impaired nerve blood flow, and ischemia (Fig. 1, black).

Glucose metabolism through the aldose reductase pathway impairs antioxidative defense directly through NADPH depletion (Hohman et al., 1997), and indirectly through osmotic stress related to sorbitol accumulation (Fig. 1, red). As mentioned above, osmotic stress may promote oxidative stress through depletion of glutathione and other putative antioxidants such as taurine (Aruoma et al., 1988), and perhaps by other yet unidentified mechanisms. The hypothesis that aldose reductase pathway activation produces mitochondrial dysfunction through osmotic stress is suggested by worsening oxidative stress when the second sorbitol dehydrogenase step in the aldose reductase pathway is blocked, leading to exaggerated sorbitol accumulation. Mitochondrial dysfunction could impair antioxidative defense by diminishing ATP for the de novo synthesis of glutathione (Hothersall et al., 1988), and by enzymes in the respiratory chain downstream from cytochrome C reductase (that generates oxygen free radicals). Aldose reductase pathway activation may also contribute to the activation of PKC that is reported in some (Inoguchi et al., 1992; Bursell et al., 1997) but not all tissues prone to diabetic complications (total PKC activity is reduced or normal rather than increased by diabetes in rat sciatic nerve) (Kim et al., 1991; Kowluru et al., 1998), but selective activation of specific isoforms in some components of the PNS has not been excluded; such cell- and isoform-specific PKC activation has been described in diabetic kidney (Koya et al., 1997). Aldose reductase pathway activation could promote de novo diacylglycerol synthesis by diverting dihydroxyacetone phosphate towards formation of α-glycerophosphate, or through osmotic stimulation of the JNK-kinase cascade. PKC activation could further exacerbate osmolyte depletion promoted by sorbitol accumulation by inhibiting the transport activity of the Na^+-*myo*-inositol (Karihaloo et al., 1997) and the Na^+-taurine (Brandsch et al., 1993) cotransporters. If activation of endoneurial or perineurial vascular PKC promoted vasoconstriction and nerve ischemia, then this would further exacerbate mitochondrial dysfunction through oxygen deprivation (Fig. 1, purple). Mitochondrial dysfunction, impaired antioxidative defense, and ischemia would all contribute further to the generation of reactive oxygen species, which would further exacerbate vasoconstriction (Fig. 1) (Low et al., 1997a,b).

6. Oxidative stress and nervous system damage

Reactive oxygen species may interact with diminished neurotrophic support in experimental diabetic neuropathy (Fig. 1, green). The PNS is subject to oxidative stress by diabetes (Low and Nickander, 1991), and neurons and the PNS are particularly vulnerable to oxidative stress (Romero et al., 1991). In addition, neurotrophic support in the PNS is reduced by diabetes (Fernyhough et al., 1995), and this reduction can be mediated by reactive oxygen species (Hounsom et al., in press). Reactive oxygen species contribute to ischemia-reperfusion injury (Chan et al., 1995). Reactive oxygen species-induced programmed cell death may share similar cell death pathways with neurotrophic withdrawal (Luo et al., 1998; Park et al., 1998), and neurotrophins may protect against reactive oxygen species-induced programmed cell death by inducing antioxidative defense mechanisms (Jackson et al., 1990a,b; Nistico et al., 1992). Recent studies have identified abundant programmed cell death in dorsal root ganglia neurons of STZ-induced diabetic rats, a finding which can be reproduced acutely by glucose infusion into non-diabetic rats (Russell et al., in press). When viewed in the context of abundant evidence of mitochondrial dysfunction in STZ-induced diabetic rats that is corrected by antioxidant therapy (Stevens et al., submitted), it is tempting to speculate that reactive oxygen species-induced mitochondrial damage in STZ-induced diabetic rats could trigger PCD through the release of such known caspace activators as mitochondrial cytochromes. This construct is further supported by recent data suggesting that antioxidant therapy may ameliorate some aspects of reduced neurotrophic support in experimental diabetic neuropathy (Garrett et al., 1997).

Thus, oxidative stress and reactive oxygen species link all of the potential initiators, encompass most of the compartments, and relate to virtually all of the physiological mediators implicated in the progressive nerve fiber dysfunction, damage and loss in diabetic peripheral neuropathy (Fig. 1). In each of these pathogenetic elements, generation of reactive oxygen species may initiate a feed-forward cycle, since oxidative stress itself impairs antioxidative defense mechanisms (Fig. 1, double-headed black arrow) (Magwere et al., 1997), resulting in a ''vicious'' cycle of metabolic damage, promoting PCD as a final common pathway.

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