PERSPECTIVE

PDE5 inhibitors promote recovery of peripheral neuropathy in diabetic mice

Diabetes mellitus affects an estimated 422 million people worldwide. Peripheral neuropathy is one of the most common and disabling complications of diabetes. There is currently no effective treatment for diabetic neuropathy, even with diligent blood glucose control, and it is thereby imperative to develop therapeutic approaches for this condition (Boucek, 2006).

Diabetic peripheral neuropathy is closely associated with vascular dysfunction, segmental demyelination and axonal degeneration of the peripheral nerve, which collectively decrease peripheral nerve conduction velocity. Diabetes induces distal axonal damage of the dorsal root ganglia (DRG) neurons, which leads to the syndromes of numbness, loss of sensation, and pain in the toes, feet and hands at the early stage of diabetic peripheral neuropathy. Decreased blood flow to peripheral nerves may result in degeneration of nerve fibers and loss of the myelin sheath. Restoring blood flow therefore improves conduction velocity in diabetic patients. Therapies targeting neurovascular function have been shown to restore nerve function in experimental diabetic peripheral neuropathy (Wang et al., 2015, 2016).

Diabetic peripheral neuropathy can develop in people with type I or type II diabetes, the latter afflicting the majority of diabetics. C57BLKS/J-$m^{+/+}Lepr^{db}$ homozygous (db/db) mice, a mouse model of type II diabetes, share a number of features with human diabetic neuropathy, such as structural, functional and biochemical alterations, and are widely used for the experimental study of diabetic peripheral neuropathy.

Phosphodiesterase-5 (PDE5) is highly specific for hydrolysis of cyclic nucleotides monophosphate, such as cyclic guanosine monophosphate (cGMP), which is a molecular messenger involved in regulation of vascular function, axon guidance, the modulation of diabetic neuropathy and pain perception (Jain et al., 2001; Patil et al., 2004; Wang et al., 2011). PDE5 inhibitors including sildenafil, tadalafil, and vardenafil, are primarily used as pharmacological agents for the treatment of erectile dysfunction (ED), but they also have a potential therapeutic application for the treatment of neurovascular dysfunction, neuroinflammatory and neurodegenerative diseases by inducing accumulation of cGMP and activation of cGMP dependent protein kinase, *e.g.*, protein kinase G (PKG), signaling pathways (Wang et al., 2011; Peixoto et al., 2015). Clinical study demonstrates that PDE5 inhibitors are safe and generally well tolerated with no serious side effects in patients. Analysis of human data from case reports show that patients with erectile dysfunction treated with sildenafil exhibit reduced diabetic peripheral neuropathy symptoms (Hackett, 2006).

Tadalafil is structurally and pharmacokinetically distinct from sildenafil and has a longer half-life of over 17 hours, and its effects persist for up to 36 hours, while sildenafil has a half-life of 4 hours (Kamenov, 2011). Tadalafil and vardenafil are equally effective in patients with diabetic neuropathy and diabetic erectile dysfunction (Kamenov, 2011). Several preclinical studies on the use of PDE5 inhibitors for the treatment of diabetic neuropathy have been published (Jain et al., 2001; Patil et al., 2004; Wang et al., 2011, 2014, 2015). The aim of this paper is to review present knowledge of the use of PDE5 inhibitors in diabetic neuropathy.

Studies of PDE5 have primarily focused on vasculature. Sildenafil improves vascular function and blood supply to the vasa neurvorum while ameliorating neurological function of neuropathy in diabetic patients and rodent models (Schafer et al., 2008). Sildenafil reduces tactile allodynia and produces peripheral antinociception in several pain models, suggesting beneficial effect on the treatment of diabetic peripheral neuropathy (Jain et al., 2001; Patil et al., 2004).

Our data showed that treatment of diabetic db/db mice, at age of 16 weeks with sildenafil significantly augments axonal outgrowth and myelination in sciatic nerve, a change associated with improvement of sciatic nerve conduction velocities and sensory function, supporting the therapeutic benefit of sildenafil on diabetic peripheral neuropathy (Wang et al., 2011).

Diabetic peripheral neuropathy is a chronic disease, and a short acting treatment may not be an optimal therapeutic approach. The considerably longer duration of action for tadalafil may permit less frequent dosing and could potentially reduce adverse effects associated with treatment. Moreover, the absorption and activity of tadalafil is unaffected by food ingestion, age, diabetes, or mild to moderate hepatic insufficiency. Also, tadalafil did not lower systemic blood pressure in clinical trials. Therefore, we investigated the therapeutic effect of tadalafil for diabetic peripheral neuropathy (Wang et al., 2016).

Administration of tadalafil every 48 hours for 8 consecutive weeks significantly improved motor and sensory conduction velocities in the sciatic nerve and peripheral thermal sensitivity in diabetic db/db mice. Tadalafil treatment also markedly increased local blood flow and the density of functional vessels in the sciatic nerve concomitantly with increased intraepidermal nerve fiber density. Tadalafil reversed the diabetes-induced reductions of axon diameter and myelin thickness in the sciatic nerve of diabetic mice (Wang et al., 2016). Thus, tadalafil treatment provides additional therapeutic opportunities for the use of multiple PDE5 inhibitors in the treatment of diabetic peripheral neuropathy. However, a direct comparison between tadalafil and sildenafil on diabetic peripheral neuropathy remains to be conducted.

Although sildenafil and tadalafil treatments are effective for improving neurological function in the early stage of diabetic peripheral neuropathy, can the therapeutic effect of PDE5 inhibitors be achieved in diabetic mice with advanced peripheral neuropathy? This information is highly clinically relevant because patients with diabetes enrolled in clinical trials often have advanced peripheral neuropathy, and induction of functional recovery in the later stage diabetic patient presents a treatment challenge. To mimic the clinical situation, we investigated the effect of sildenafil on advanced peripheral neuropathy in middle aged diabetic db/db mice.

Treatment of db/db mice with sildenafil starting at age of 36 weeks daily for 8 consecutive weeks enhanced regional blood flow and functional vascular density in the sciatic nerve tissue, increased intraepidermal nerve density and sciatic nerve myelin thickness, improved motor and sensory conduction velocities in the sciatic nerve and improved the thermal sensitivity of diabetic mice. These findings suggest that sildenafil is effective for treating 36 week old db/db mice with late-stage peripheral neuropathy (Wang et al., 2015). However, compared to the treatment of early-stage diabetic peripheral neuropathy, sildenafil treatment did not significantly improve sensory function until 6 weeks after the initial treatment and did not robustly augment axon diameter of sciatic nerves in advanced diabetic peripheral neuropathy (Wang et al., 2015). These results suggest that the success of axonal remodeling may be dependent on the intensity and stages of damage and on therapy duration. Axonal damage induced by diabetes may be irreversible at an advanced stage. An 8 week course of treatment may not be sufficient to induce a significant increase in the axonal diameter of the sciatic nerve. Middle-aged diabetic mice with long term neuropathy may have decreased ability to respond to sildenafil treatment. Our data are consistent with preclinical and clinical studies demonstrating that the late phases of diabetic neuropathy are poorly reversible, and early intervention is an important determinant of outcome in diabetic neuropathy (Boucek, 2006). We acknowledge that functional benefit likely derives from an interaction of multiple changes of tissue including, axonal outgrowth, myelination and vascular plasticity, and multiple signaling pathways may be involved in

PDE5 inhibitor–enhanced neurovascular remodeling.

To investigate the molecular mechanisms that mediate PDE5 inhibitor–enhanced axonal outgrowth and myelination in diabetic mice, the effect of sildenafil on expression of PDE5, cGMP, and activation of cGMP/PKG signaling pathways was examined (Wang et al., 2011). Diabetic db/db mice upregulated PDE5 expression in the sciatic nerve. Sildenafil suppressed PDE5, thereby increasing cGMP levels and activating the cGMP/PKG signaling pathway that mediates axonal remodeling, leading to improvement of neurological function (Wang et al., 2011).

Angiopoietins (Ang) and their receptor Tie-2 signaling pathway have multiple effects on neurovascular remodeling that may contribute to improved neurological function. Ang1 promotes vascular stabilization and maturation, and, Ang2 acts as a partial agonist or antagonist of Ang1 signaling. Ang1 and Ang2 also promote neurite outgrowth in DRG neurons. Our in vivo and in vitro data indicate that hyperglycemia considerably downregulates Ang1 and upregulates Ang2 in endothelial cells and Schwann cells of the sciatic nerve. Sildenafil reverses the expressions of Ang1 and Ang2 and promotes vascular function and axonal remodeling in the sciatic nerve of diabetic peripheral neuropathy. More importantly, blockage of Ang/Tie2 signaling attenuates the effect of sildenafil on endothelial cells, Schwann cells and DRG neurons under hyperglycemia condition (Wang et al., 2015). These data support the hypothesis that the Ang/Tie2 signaling pathway mediates sildenafil-improved neurovascular function.

PDE5 inhibitor-induced activation of the cGMP/PKG and Ang/Tie2 signaling pathways promotes neurovascular remodeling both directly through these signaling pathways to ameliorate neurovascular function, and indirectly via endothelial cells and Schwann cells, which produce neurotrophic factors and provide a permissive restorative microenvironment in the sciatic nerve. Both direct and indirect approaches, in concert, improve neurological function of diabetic peripheral neuropathy.

Garcia et al. reported that PDE5 inhibitors attenuate the production of damaging factors and up-regulate the expression of beneficial factors in the peripheral nerve, thereby promoting a neuroprotective environment favoring neuron survival and the amelioration of neuropathic pain (Garcia et al., 2014). BDNF, NGF and PDGF-C are neurotrophic factors that not only promote vascular growth and maturation, but also directly regulate axonal remodeling by binding to their receptors, TrkB, TrkA and PDGF-α/β, respectively. These factors appear to be important components of neurovascular interaction and play significant roles in the treatment of diabetic neuropathy (Wang et al., 2011, 2016). Our studies show that hyperglycemia reduced BDNF, NGF and PDGF-C proteins in the sciatic nerve tissue, whereas PDE5 inhibitor treatment increased the expression of these proteins, and thereby promotes neurovascular remodeling in diabetic peripheral neuropathy.

How do PDE5 inhibitors impact multiple signaling pathways? To obtain insight into the PDE5 inhibitor–mediated activation of molecular pathways, we investigated the effects of PDE5 inhibitors on microRNAs (miRNAs) that mediate gene expression through mRNA destabilization and/or translational repression, and are involved in biological function of diabetic peripheral neuropathy. miRNAs are promising potential biomarkers and therapeutic targets in clinical pain disorders (Wang et al., 2014). Sildenafil promoted changes in the gene expression of pro-inflammatory and anti-inflammatory cytokines (Garcia et al., 2014). We found that hyperglycemia downregulated miR-146a and elevated its target proteins IRAK1 and TRAF6, leading to reduction of axonal outgrowth and apoptosis in DRG neurons. Sildenafil reversed the effect of hyperglycemia on miR-146a and its target proteins, which were associated with promoting axonal outgrowth and suppressing apoptosis of DRG neurons (Wang et al., 2014). These data suggest that miR-146a plays an important role in mediating DRG neuron axonal outgrowth and apoptosis under hyperglycemia conditions, and miR-146a may serve as a common therapeutic mechanism of PDE5 inhibitors for diabetic peripheral neuropathy. We are aware that miR-146a is not alone in driving neurological recovery, and there are multiple sets of miRNAs affected by PDE5 inhibitors that enhance neurological recovery.

Our preclinical studies provide evidence that the PDE5 inhibitor treatment augments vascular function and axonal remodeling, changes that are associated with improved neurological functional outcome in early and advanced stage diabetic mice with peripheral neuropathy, but that do not affect blood glucose levels and animal body weight in diabetic mice. The cGMP/PKG and Ang1/Tie2 signaling pathways and BDNF/NGF/PDGF factors likely mediate the therapeutic effect of PDE5 inhibitors on diabetic peripheral neuropathy. Thus, PDE5 inhibitors, which are FDA approved medications, are potent neurorestorative agents and could have potential clinical application for patients with diabetic peripheral neuropathy.

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