REVIEW ARTICLE

Touching Greater Horizons in the Management of Diabetic Neuropathy: Epalrestat in Advanced Role

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ABSTRACT

The major factor contributing to the pathogenesis of type 2 diabetes and its various complications is insulin resistance (IR). The lesser known and interesting element that has been unfolding is about the existence of IR in neurons associated with disrupted phosphatidylinositol 3-kinase/protein kinase B (PI3k/Akt) pathway with subsequent adverse effect on nerve function. Hyperactivity of polyol pathway is identified as the prime convincing mechanism elucidating the metabolic link between hyperglycemia and nerve dysfunction. Additionally, in the polyol trail, reduction in glutathione leads to redox imbalance and eventually neuropathy.

Epalrestat, the aldose reductase inhibitor (ARI), has been extensively evaluated for its efficacy and safety in the treatment of diabetic neuropathy. Addition of methylcobalamin to Epalrestat therapy has shown faster and better resolution of neuropathy. Epalrestat-induced activation of PI3k/Akt signaling pathway promotes proliferation and migration of endothelial cells, with potential benefits of improved blood supply to damaged nerves. Further evidences reveal upregulation of nerve growth factor (NGF) by Epalrestat that has resulted in wound healing in diabetic patients. Increase in nerve fiber length is the other compatible finding with Epalrestat therapy in patients suffering from diabetic neuropathy. Moreover, Epalrestat has demonstrated significant protection against oxidative stress via induction of glutathione biosynthesis.

Keywords: Diabetic neuropathy, Epalrestat, Glutathione, Methylcobalamin, Nerve growth factor, Neuronal insulin resistance, Oxidative stress, Phosphatidylinositol 3-kinase/protein kinase B (PI3k/Akt) pathway.

INTRODUCTION

A recent report by the International Diabetes Federation reveals that globally 415 million people are suffering from diabetes, and a whopping rise to 642 million is estimated

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by 2040.[1] In patients with type 2 diabetes mellitus, hyperglycemia leads to secondary biochemical abnormalities in various target tissues, which are followed by alteration in their functions, formation of structural lesions, and finally, clinical aberrations. Neuropathy, the microvascular diabetic complication, is manifested in about 70% of the patients.[2] This debilitating condition of nerve damage is associated with extremely distressing symptoms like stabbing pain, pins and needles, burning sensation, tingling, and numbness. These sensory deficits generally worsen from paresthesia to anesthesia. Diabetic peripheral neuropathy is the major predisposing factor for foot ulceration and infections. Nontraumatic lower limb amputations occur in more than 60% of patients with diabetes mellitus.[3]

Increased insulin resistance (IR) and impaired insulin secretion are the main pathophysiological features of type 2 diabetes that jointly contribute to the development of this disease and its sequel in the form of microvascular and macrovascular complications. As IR worsens, more defects occur in insulin secretion that results in increased hepatic glucose output and inefficient utilization of glucose by skeletal muscle and adipose tissue. This compromised insulin signaling in these tissues heightened the risk of neuropathy, nephropathy, retinopathy, and macrovascular complications associated with diabetes.

INSULIN RESISTANCE IN NEURONS

Accumulating evidences suggest that insulin exerts pleiotropic effects in neurons, including the regulation of neuronal proliferation, apoptosis, synaptic transmission, neuronal degeneration, and learning. Insulin is considered a potent neurotrophic factor for maintaining neuronal functioning. Insulin shares a variety of intracellular signaling components with nerve growth factor (NGF) for sustaining nerve health. An interesting fact related to the role of IR in neurons has been discussed in various articles and is gaining attention. Though neurons are not insulin dependent, they respond to neurotrophic action of insulin. It is observed that insulin receptors and insulin signaling are not exclusively restricted to metabolically active tissues dependent on insulin but are also observed in neuronal cells.[4]

Neurons also can develop IR, which in turn results in injury to the peripheral and central nervous systems

and is the probable cause for the development of diabetic neuropathy. After the development of neuronal IR, the response to insulin and NGF seems to be reduced, which increases vulnerability to neuronal damage. Thus, research suggests that neuronal IR spurs the development of neurological diseases (diabetic neuropathy, Alzheimer's disease, etc.) and metabolic insults, such as hyperglycemia both contributing to the development of diabetic neuropathy.[5]

ROLE OF PI3K/AKT IN PHYSIOLOGY

The prototype of hormonal function requires a cellular source of signals and a definite target able to interpret and respond to the stimulation. Multiple signaling mechanisms are known to detect extracellular signals, deliver intracellular amplification, and generate explicit responses. A major signaling cascade involved in several physiological roles is phosphatidylinositol 3-kinase/protein kinase B (PI3k/Akt) pathway, which belongs to the kinase family and exists in almost all the mammalian cells. The growing number of hormones that relay signals by activating PI3k suggests not only multiple roles of these enzymes in the regulation of different physiological responses but also a way by which common reactions can be stimulated by different inputs. Several PI3k products exert their large number of biological roles by their ability to function as docking sites for proteins that contain specific binding domains.[6-8]

One of the most studied signaling events triggered by activation of PI3k is recruitment of Akt. Phosphatidylinositol 3-kinase assists in the conversion from phosphatidylinositol-4,5-biphosphate to the second messenger phosphatidylinositol-3,4,5-triphosphate, which triggers the phosphorylation of Akt. Activated Akt is able to phosphorylate a vast number of proteins, thus representing a key effector of PI3k signaling. This pathway is involved in various vital processes, which sets off a sequence of intracellular responses for growth, survival, and metabolism in numerous cellular systems.[6-9]

NEUROPATHOLOGY VIA DISRUPTION OF PI3K/AKT PATHWAY

Significant investigations have clarified the role of PI3k/Akt pathway as a crucial component for both neuronal survival and neuritogenesis; it structures as an important mediator of insulin signaling downstream of the insulin receptor. Emphasis on the involvement of PI3k/Akt signaling in diabetes and its allied complications has been described in several experiments. The disruption of PI3k/Akt signaling is related to the development of IR, and appropriate functioning of this pathway is critical for elucidating the metabolic effects of insulin.[10]

Insulin activates PI3k and induces phosphorylation of Akt. This activated Akt is further responsible for carrying out the neurotrophic effect of insulin in neurons by stimulating specific proteins. The insulin–PI3k/Akt pathway plays a vital role in regulation of neuronal functioning.[11] It has been resolved in many experimental studies that insulin-mediated activation of this pathway is one of the most important aspects associated with diabetic neuropathy. Also, insulin-like growth factor is involved in initiating this pathway for sustenance of neuronal health.[12]

## ROLE OF NERVE GROWTH FACTOR IN MAINTAINING NEURONAL HEALTH

Neurotrophins have remarkable effects on neurite outgrowth and reduces neuronal degeneration. The biological effects of neurotrophins are signaling cascade. Nerve growth factor is an important neurotrophic agent involved in growth, maintenance, and survival of nerve cells. It has been demonstrated that NGF reduces neuronal degeneration and promotes neuritogenesis by activating PI3k/Akt pathway.[13]

Nerve growth factor-induced neuronal cell survival requires functional PI3k/Akt pathway. Reduction in NGF concentration in neurons is possibly a major factor responsible for the development of diabetic neuropathy.[14] There are evidences that NGF concentration is reduced in wounds of diabetic patients.[15] Thus, elevation in NGF level leads to improvement in diabetic wound healing as well as nerve damage. Additionally, disruption of PI3k/Akt pathway could hamper NGF activity (Flow Chart 1).[16]

INFLUENCE OF POLYOL PATHWAY AND OXIDATIVE STRESS IN DIABETIC NEUROPATHY

The most noticeable abnormality in diabetes relates to diversion of glucose metabolism from oxidative to reductive pathways as evidenced in polyol mechanism. Under normal circumstances, glucose is primarily oxidized to carbon dioxide and water for energy generation in the form of adenosine triphosphate (ATP). In the presence of hyperglycemia, excess glucose enters the nerves since

Flow Chart 1: Effect of IR on neuronal functioning
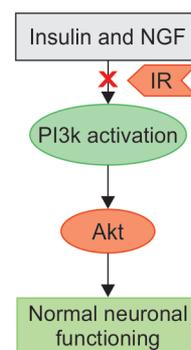
its transport is neither rate limiting for metabolism nor insulin dependent. The glycolytic pathways become saturated leading to increased flux of glucose through polyol pathway as an alternative to glucose metabolism.

Since its discovery, polyol pathway has grabbed attention due to its conspicuous role in the development of neuropathy. The pathobiology of neuropathy includes the overtriggering of polyol pathway in hyperglycemia (Flow Chart 2). Aldose reductase (AR) is the key enzyme responsible for the conversion of glucose to sorbitol in this pathway. In 1956, Hers et al first identified AR as the major propagator of this pathway. Later, several studies revealed that hyperactivity of polyol pathway induces accumulation of sorbitol in tissues.[17]

In healthy individuals, this pathway converts less than 3% of glucose into sorbitol, but in hyperglycemic condition up to 30% of glucose gets reduced to sorbitol. Accumulation of sorbitol, being an osmolyte, leads to osmosis in the nerves, which causes neuronal edema. As a compensatory mechanism, deposition of sorbitol results in depletion of other osmolytes like myoinositol. The loss of myoinositol leads to alteration in phosphoinositide metabolism, with decreased availability of diacylglycerol, which contributes to reduction in neuronal sodium-potassium ATPase (Na^+-K^+-ATPase) activity. The altered Na^+-K^+-ATPase activity would lead to impaired neural signaling and nerve conduction defect. Accumulation of sorbitol into the tissues increases the susceptibility to oxidative stress by reduction in nicotinamide adenine dinucleotide phosphate (NADPH) levels. Nicotinamide adenine dinucleotide phosphate is required as a cofactor for the formation of antioxidant, i.e., glutathione (GSH).[18-20]

Glutathione is an endogenous antioxidant composed of amino acids glutamine, cysteine, and glycine.

Flow Chart 2: Impact of hyperactivity of polyol pathway

AR: Aldose reductase; SDH: Sorbitol dehydrogenase; NADPH: Nicotinamide adenine dinucleotide phosphate; AGEs: Advanced glycation end products

It is synthesized by consecutive actions of two enzymes gamma-glutamylcysteine synthetase (γ-GCS) and GSH synthetase. Research has highlighted the beneficial roles of GSH in several molecular systems. Intracellular GSH can be present in reduced as well as oxidized form. Glutathione is utilized to quench the circulating reactive oxygen species (ROS) or free radicals in the body. The detoxification of free radicals is catalyzed by GSH reductase in the presence of NADPH. Thus, low concentration of NADPH would lead to decreased efficacy of GSH as an antioxidant. Normally, a balance is maintained between detoxification and generation of ROS. The imbalance between GSH concentration and oxidative stress seems to be responsible for nerve damage leading to diabetic neuropathy.[21,22]
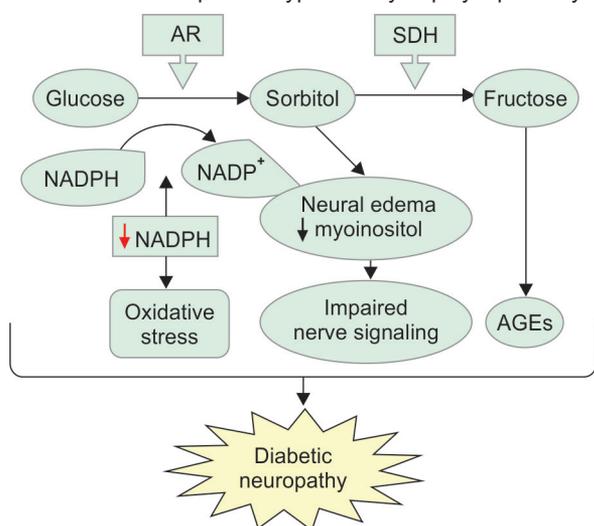
Regarding GSH synthesis, it has been indicated that activation of the PI3k/Akt pathway and the translocation of nuclear factor erythroid 2-related factor-2 (Nrf2) mediates the positive regulatory signal. Phosphatidylinositol 3-kinase/Akt is a major pathway for the Nrf2-mediated regulation in synthesis of GSH. High levels of GSH which are important as a defense against the generation of oxidative stress relates to the activation of PI3k/Akt signaling cascade.[18,23]

DRUGS AFFECTING PATHOGENESIS OF DIABETIC NEUROPATHY

Tight glycemic control is vital to prevent the development of neuropathy and further worsening of the condition. However, over a period of time, glycemic control declines due to the progressive nature of diabetes leading to increased risk of complications. Ameliorating the neuropathy symptoms like pain, numbness, tingling, burning, etc., is extremely important. Neuroanalgesics like tricyclic antidepressants, selective serotonin and norepinephrine reuptake inhibitors, calcium channel alpha 2 delta ligands, opioids, Lidocaine patches, etc., are often taken into consideration to improve physical functioning in patients complaining of neuropathic pain. Several other agents like Alpha lipoic acid, Gamma linolenic acid, Pyridoxine, Benfotiamine, Nicotinamide, Methylcobalamin, etc., have been considered in the management of diabetic neuropathy. Clinical trials have established the beneficial effects of these neurotrophic agents in diabetic neuropathy since many years.

Efficacy of Methylcobalamin in diabetic neuropathy has been demonstrated in several clinical studies.[24,25] Methylcobalamin is active in spinal fluid, and due to this property, it is able to regenerate the damaged neurons and restore the normal functions. Methylcobalamin provides symptomatic relief by improving nerve conduction velocity impaired in diabetic neuropathy.[26,27] It has been shown that exogenous administration of methylcobalamin delays the onset of diabetic neuropathy.[28] A relatively new class

of drugs, i.e., aldose reductase inhibitor (ARI), has also gained attention worldwide for its efficacy in delaying the development of diabetic neuropathy and improving the associated symptoms. This class specifically targets polyol pathway – the prime pathogenic mechanism of diabetic neuropathy. The efficacy of ARI like Epalrestat in improving diabetic neuropathy has been widely documented.

EPALRESTAT: A POTENTIAL ARI FOR THE TREATMENT OF DIABETIC NEUROPATHY

Epalrestat is a carboxylic acid derivative that has been widely documented in the management of diabetic neuropathy.[29-32] Epalrestat easily penetrates into the neural tissue and potently inhibits the enzyme AR resulting in decreased sorbitol accumulation. Epalrestat has remarkably shown to improve the neuropathy symptoms like loss of sensation and thermal sensitivity, burning sensation, numbness, muscle cramps, pain, weakness, dizziness, and nerve conduction velocity in patients suffering from diabetic neuropathy.[26,29,31] Various studies have demonstrated novel mechanisms of Epalrestat likely to have benefits in managing diabetic neuropathy (Flow Chart 3).

Effect of Epalrestat on PI3k/Akt Signaling

Cell proliferation or angiogenesis in a balanced way is an important aspect for normal growth. Patients with type 2 diabetes mellitus are prone to have ischemic vascular diseases and impaired wound healing due to defective angiogenesis. Epalrestat has been reported to activate PI3k/Akt pathway in the endothelial cells leading to increased endothelial cell proliferation.[30] Maintenance of adequate blood supply to a damaged area assists in earlier regeneration of nerves.[33] The angiogenic effect of Epalrestat helps in reducing the risk of developing neuropathy. Thus, activation of PI3k/Akt seems to be a plausible mechanism for faster wound healing and neuronal growth.

Effect of Epalrestat on GSH Levels

Epalrestat has been reported to increase the biosynthesis of GSH, and pretreatment with Epalrestat in Schwann cells has shown to have reduced toxicity. Epalrestat increases GSH by elevated expression of Nrf2, which is a key transcriptor in regulating γ-GCS.[34] Also, Epalrestat by inhibiting the enzyme AR increases the availability of cofactor NADPH for GSH reductase, which is critical for maintaining the levels of GSH. Epalrestat by increasing the intracellular concentration of GSH enhances the resistance toward redox imbalance, which aids strongly in reducing the development of diabetic neuropathy.

Effect of Epalrestat on NGF Concentration

It has been found that the concentration of NGF is less in wounds of diabetics, and several studies have demonstrated the association of high NGF levels with better wound healing. Treatment with Epalrestat has shown to increase the messenger ribonucleic acid expression of NGF.[15] This elevates NGF concentration and improves wound healing. Thus, Epalrestat has the potential for improving diabetic wound healing through upregulation of NGF concentration.

A clinical study has revealed that treatment with Epalrestat increases the nerve fiber length.[35] The possible mechanism for this effect is increase in the NGF concentration which leads to nerve regeneration. This regeneration of nerve fiber aids in delaying the onset of diabetic neuropathy and improving the neuropathy symptoms.

## COMBINATION THERAPY FOR THE MANAGEMENT OF DIABETIC NEUROPATHY

More than one agent is frequently employed in the management of neuropathy associated with diabetes. Clinical trials have established the efficacy of

Flow Chart 3: Beneficial actions of Epalrestat in diabetic neuropathy
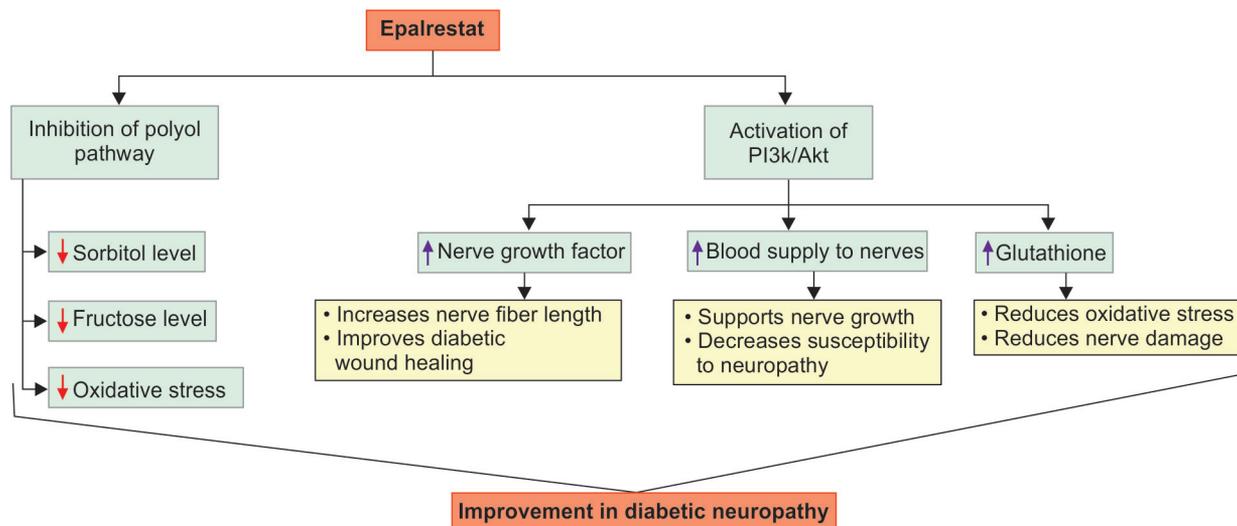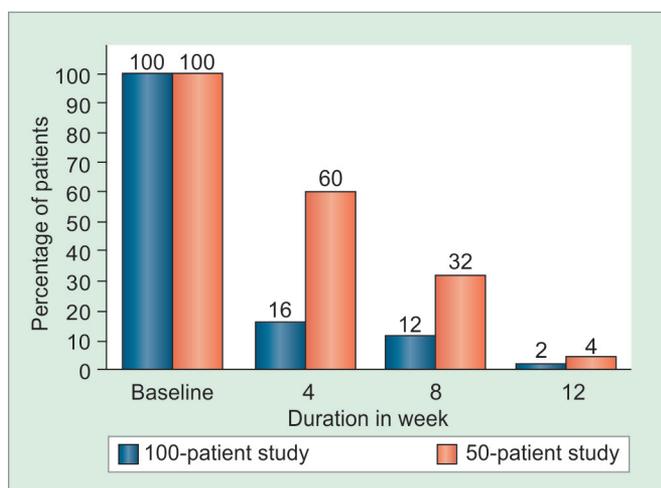
Graph 1: Percentage of patients experiencing reduction in neuropathy symptoms
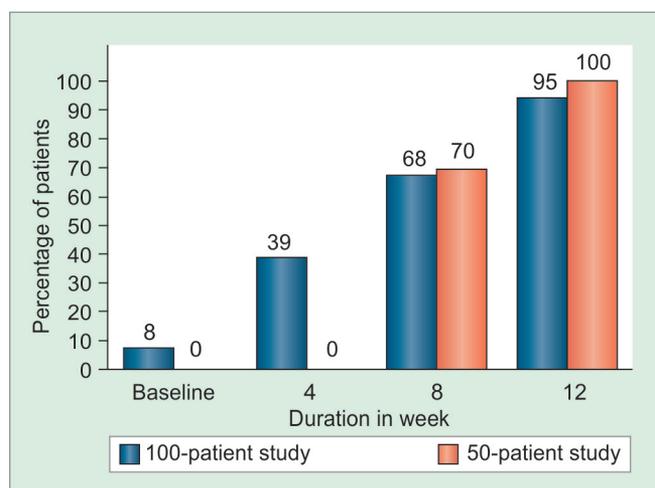
Graph 2: Percentage of patients with normal tendon reflex

different combinations including Pregabalin, Epalrestat, Methylcobalamin, Alpha lipoic acid, Benfotiamine, Gabapentin, Venlafaxine, Amitriptyline, Nortriptyline, Morphine, and Tramadol.[36-39] Epalrestat and Methylcobalamin in combination have shown to elucidate more beneficial effects than administering them alone. The synergistic effect of these agents results in better resolution of neuropathic symptoms.

The postmarketing surveillance involving 1,327 neuropathic patients revealed that the combination of Pregabalin and Methylcobalamin was well tolerated and efficacious in reducing neuropathic pain.[38] A clinical trial demonstrated that the cotherapy of Gabapentin and Morphine had significant efficacy in reducing pain involving neuropathic patients.[40] Combined treatment with Gabapentin and Nortriptyline showed significant improvement in neuropathic pain as compared with either drug administered alone.[39]

A 12-week randomized comparative study in 220 patients with diabetic neuropathy found Epalrestat and Methylcobalamin combination was associated with faster and better symptomatic relief than Epalrestat alone. The study concluded that the combination of Epalrestat and Methylcobalamin is a better option for the treatment of diabetic neuropathy.[31] Additionally, the postmarketing surveillance on fixed dose combination of Epalrestat and Methylcobalamin in 500 diabetic patients suffering from peripheral neuropathy confirmed the safety and efficacy of this combination therapy in the management of diabetic neuropathy.[32]

The earlier observations were corroborated in our two recent studies conducted on 100 and 50 neuropathic patients respectively. These patients were treated with fixed dose combination containing Epalrestat 50 mg and Methylcobalamin 500 µg administered thrice daily for 12 weeks. Reduction in neuropathy symptoms was observed in 98 and 96% (Graph 1) of patients respectively, in these studies. In 100-patient study, 95% of cases experienced normal tendon reflex, whereas in the 50-patient study, all the cases achieved normalization of tendon reflex at the end of treatment (Graph 2). Significant resolution of neuropathic pain was observed in both the studies. The efficacy of treatment was found to be very good in more than 85% of patients; tolerability was observed to be good to excellent according to physicians as well as patients. No major adverse events were evidenced in both the studies.

CONCLUSION

The understanding about the development of neuropathy is gaining importance from a novel perspective pertaining to IR in neurons. The neuronal IR hampers PI3k/Akt-mediated neurotrophic effect of NGF and also adversely affects the biosynthesis of GSH. The ARI Epalrestat activates PI3k/Akt pathway, increases NGF, and enhances GSH levels with potential benefits in improving diabetic neuropathy.

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