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From Animal Models to Clinical Practicality: Lessons Learned from Current Translational Progress of Diabetic Peripheral Neuropathy

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1. Introduction

1.1. Overview of DPN

In diabetes mellitus, nerves and their supporting cells are subjected to prolonged hyperglycemia and metabolic disturbances and this culminates in reversible/irreversible nervous system dysfunction and damage, namely diabetic peripheral neuropathy (DPN). Due to the varying compositions and extents of neurological involvements, it is difficult to obtain accurate and thorough prevalence estimates of DPN, rendering this microvascular complication vastly underdiagnosed and undertreated [1-4]. According to American Diabetes Association, DPN occurs to 60-70% of diabetic individuals [5] and represents the leading cause of peripheral neuropathies among all cases [6, 7]. As the incidence of diabetes is approaching global epidemic level, its neurological consequences are estimated to affect some $300 million people worldwide [8] and costs 15 billion dollars on annual healthcare expenditures in the U.S. alone [9].

1.1.1. A Complex natural history

Because diverse anatomic distributions and fiber types may be differentially affected in patients with diabetes, the disease manifestations, courses and pathologies of clinical and subclinical DPN are rather heterogeneous and encompass a broad spectrum [1, 10, 11]. Additionally, dietary influences, risk covariates, genetic and phenotypic multiplicity further perplex the definition, diagnosis, classification and natural history of DPN [6, 10, 12, 13]. Current consensus divides diabetes-associated somatic neuropathic syndromes into the

focal/multifocal and diffuse/generalized neuropathies [6, 14]. The first category comprises a group of asymmetrical, acute-in-onset and self-limited single lesion(s) of nerve injury or impairment largely resulting from the increased vulnerability of diabetic nerves to mechanical insults (Carpal Tunnel Syndrome) (reviewed in 15). Such mononeuropathies occur idiopathically and only become a clinical problem in association with aging in 5-10% of those affected. Therefore, focal neuropathies are not extensively covered in this chapter [16]. The rest of the patients frequently develop diffuse neuropathies characterized by symmetrical distribution, insidious onset and chronic progression. In particular, a distal symmetrical sensorimotor polyneuropathy accounts for 90% of all DPN diagnoses in type 1 and type 2 diabetics and affects all types of peripheral sensory and motor fibers in a temporally non-uniform manner [6, 17].

Symptoms begin with prickling, tingling, numbness, paresthesia, dysesthesia and various qualities of pain associated with small sensory fibers at the very distal end (toes) of lower extremities [1, 18]. Presence of the above symptoms together with abnormal nociceptive response of epidermal C and A-δ fibers to pain/temperature (as revealed by clinical examination) constitute the diagnosis of small fiber sensory neuropathy, which produces both painful and insensate phenotypes [19]. Painful diabetic neuropathy is a prominent, distressing and chronic experience in at least 10-30% of DPN populations [20, 21]. Its occurrence does not necessarily correlate with impairment in electrophysiological or quantitative sensory testing (QST). Some have suggested pain to reflect the pathobiological changes of serum glucose level at least in individuals with pre- or recent diagnosis. Consistent with this notion, severe neuropathic pain often presents as a typical feature in acute reversible sensory/hyperglycemic neuropathy and its onset and remission following glycemic control can be indicative of spontaneous repair of nerve damage in the early phase of DPN [1, 10, 22, 23]. Pain in many diabetics may persist, however, only to be alleviated as progressive and irreversible nerve deterioration and loss of thermal sensitivity take place [10, 21]. Large myelinated sensory fibers that innervate the dermis, such as Aβ, also become involved later on, leading to impaired proprioception, vibration and tactile detection, and mechanical hypoalgesia [19]. Following this "stocking-glove", length-dependent and dying-back evolvement, neurodegeneration gradually proceeds to proximal muscle sensory and motor nerves. Its presence manifests in neurological testings as reduced nerve impulse conductions, diminished ankle tendon reflex, unsteadiness and muscle weakness [1, 24].

Both the absence of protective sensory response and motor coordination predispose neuropathic foot to impaired wound healing and gangrenous ulceration—often ensued by limb amputation in severe and/or advanced cases [25, 26]. This traumatic procedure is performed on approximately 100,000 Americans every year and is a major attributing factor for diabetes-related hospital bed occupancy and medical expenses [27]. Although symptomatic motor deficits only appear in later stages of DPN [25], motor denervation and distal atrophy can increase the rate of fractures by causing repetitive minor trauma or falls [24, 28]. Other unusual but highly disabling late sequelae of DPN include limb ischemia and joint deformity [6]; the latter also being termed Charcot's neuroarthropathy or Charcot's joints [1]. In addition to significant morbidities, several separate cohort studies provided evidence that DPN [29],

diabetic foot ulcers [30] and increased toe vibration perception threshold (VPT) [31] are all independent risk factors for mortality. Overall, neuropathic pain, foot complication as well as various associated psychosocial comorbidities inflict a significant diminution on the quality and duration of life of individuals affected by DPN, which in turn is raising an escalating health, social and economic problem in both developed and developing countries [4, 14].

1.2. A medical challenge

Unfortunately, current therapy for DPN is far from effective and at best only delays the onset and/or progression of the disease via tight glucose control, the only established means for managing diabetic complications in the U.S. Several large-scale, multicenter and landmark clinical studies, including Diabetes Control and Complication Trial, provided irrefutable evidence that chronic hyperglycemia is a leading factor in the etiology and treatment of DPN [32-36]. However, euglycemia cannot always be achieved through aggressive insulin therapy or other anti-diabetic agents. Even with near normoglycemic control, a substantial proportion of patients still suffer the debilitating neurotoxic consequences of diabetes [34]. On the other hand, some with poor glucose control are spared from clinically evident signs and symptoms of neuropathy for a long time after diagnosis [37-39]. Thus, other etiological factors independent of hyperglycemia are likely to be involved in the development of DPN. Data from a number of prospective, observational studies suggested that older age, longer diabetes duration, genetic polymorphism, presence of cardiovascular disease markers, malnutrition, presence of other microvascular complications, alcohol and tobacco consumption, and higher constitutional indexes (e.g. weight and height) interact with diabetes and make for strong predictors of neurological decline [13, 32, 40-42]. Targeting some of these modifiable risk factors in addition to glycemia may improve the management of DPN.

Meanwhile, enormous efforts have been devoted to understanding and intervening with the molecular and biochemical processes linking the metabolic disturbances to sensorimotor deficits by studying diabetic animal models. In return, nearly 2,200 articles were published in PubMed central and at least 100 clinical trials were reported evaluating the efficacy of a number of pharmacological agents; the majority of them are designed to inhibit specific pathogenic mechanisms identified by these experimental approaches. Candidate agents have included aldose reductase inhibitors, AGE inhibitors, γ-linolenic acid, α-lipoic acid, vasodilators, nerve growth factor, protein kinase Cβ inhibitors, and vascular endothelial growth factor. Notwithstanding a fruitful of knowledge and promising results in animals, none has translated into definitive clinical success (Figure 1). While the notorious biochemical heterogeneity and temporal non-uniformity of the disease processes among and even within individuals can take much of the blame, investigators must take into serious consideration the marked differences between animals and humans, which may substantially impair the application of experimental data to clinical settings. The following sections of this chapter describe the clinical outcomes of these pathogenetic treatments that put previous observations generated by animal studies into perspective, and discuss the molecular, cellular and physiological roots underlying the limited translation.
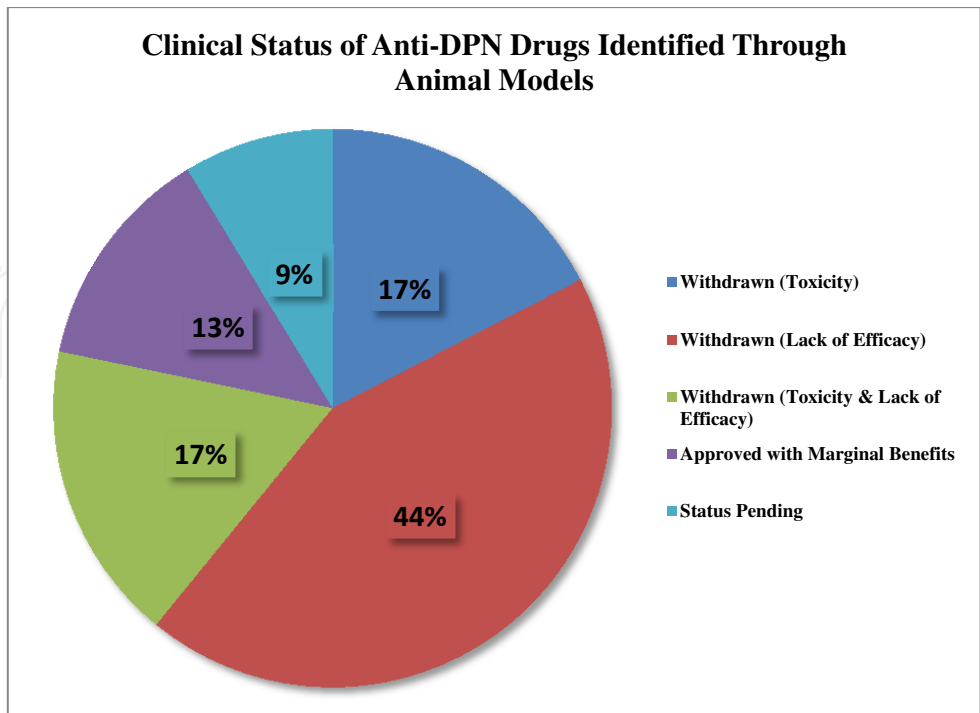
Figure 1. Summary of Current Clinical Status of Anti-DPN Drugs Developed via Animal Models. Data are generated from published experimental and clinical results to date on pharmacological agents (a total of 23 drugs) targeting pathogenetic mechanisms listed in but not limited to section 2.

2. Pharmacological management of DPN via targeting pathogenetic mechanisms: From animal models to clinical practice

2.1. Managing metabolic derangements

2.1.1. Polyol pathway and aldose reductase inhibitors

Polyol pathway arose as a plausible link of glucose dismetabolism to DPN in middle 1960s [43] and has received much interest due to the strong evidence accumulating from experimental diabetic rats [44]. Two consecutive oxidoreductive reactions essentially constitute the polyol pathway: the rate-limiting NADPH-dependent aldose reductase (AR) reduces glucose to sorbitol, which then becomes the substrate for NAD^+-dependent sorbitol dehydrogenase (SDH) and oxidized into fructose. Although AR has a high K_M for glucose under the physiological condition, hyperglysolia (high intracellular glucose concentration) can excessively activate this enzyme resulting in a nearly 4-fold induction in glucose disposal through this pathway in human erythrocytes [45, 46]. Because these polyhydroxylated alcohols have little transmembrane diffusibility, their retention within ocular lens fibrils of hyperglycemic rats or rabbits was proposed to cause hyperosmotic perturbation of intracellular metabolites, electrolytes and other osmolytes and subsequent hydropic cataractogenesis as observed. All

of these were preventable and reversible by blocking AR [47-51]. In mice, transgenic overexpression of the gene encoding human AR (hAR) in lens epithelia submitted these cataract-resistant animals to sugar-induced polyol deposit and cataract formation, which became more acute when coupled with genetic SDH deficiency [52]. Studies of type 1 and type 2 diabetes models, including alloxan- and streptozotocin (STZ)-induced diabetic rats and leptin-deficient *ob/ob* mice, soon confirmed a significant elevation of sorbitol and fructose in sciatic nerves, dorsal root ganglia (DRGs) and spinal cord. This correlated with nerve/axonal conduction and transport deficiencies, loss of intraepidermal nerve fibers, increased neural and endoneurial oxidative-nitrosative stress as well as thermal hypoalgesia and tactile allodynia [43, 53-57]. A "polyol hypothesis" derived from diabetic lens was thus propelled to the pathogenesis of DPN [47]. In keeping with this notion, AR inhibitors that reduce nerve polyol levels showed remarkable preservation of nerve structure and function in rats with either spontaneous or chemical-induced diabetes [53, 58-60]. Systemic hAR overexpression combined with STZ-induced diabetes led to an exacerbated but AR inhibitor-preventable peripheral nerve sorbitol and fructose buildup, electroactivity suppression and myelinated fiber atrophy [61]. A similar biochemical and electrophysiological but not morphological abnormality was obtained with Schwann cell (SC)-targeted hAR transgenic mice, indicating that SC AR hyperactivity contributes to many, though not all pathological change of DPN [62]. Conversely, AR-knockout mice showed no obvious sorbitol accumulation, conduction slowing, oxidative stress, or stress kinase activation. Additionally, there were fewer loss of sural nerve fibers in AR-deficient mice compared to wild-types (WTs) [63]. Since galactose has approximately 4 times higher affinity for AR than glucose [64] and its reduction product galactitol is poorly disposed, galactose-rich diet was used as a popular substitute for classical hyperglycemic models to exemplify and examine the role of excessive polyalcohol formation in the genesis of diabetic cataract and neuropathy [47]. Along the line with "aldo-osmotic theory", galactosemic rodents that accrue much greater level of this alternate AR metabolite also exhibit similar and sometimes more severe electrophysiological, anatomical and biochemical defects that are seen diabetic models [65-67]. However, galactosemia is a rare metabolic condition in humans (less than 0.002% of the population) [68] and the galactosemic lens and nerves often manifest functional and structural lesions resulting from acute and exaggerated galactitol intoxication that differ from those of diabetic cataract and neuropathy [47, 69-71]. Hence, galactose-fed animals are neither appropriate models for studying diabetic complications nor good replacements for characterizing the pathogenetic involvement of sorbitol pathway in these conditions. Other studies further revealed that neither the morphometrical [59] nor functional indices in DPN correlate with the tissue sorbitol content [72, 73]. Instead, nerve myo-inositol content is more closely related to the neurophysiological function according to most reports. Depletion of cytoplasmic myo-inositol, protein kinase C activation and tubulin/Na^+/K^+-ATPase complex formation were proposed mechanisms that mediate polyol pathway overflow-induced impairment of Na^+/K^+-ATPase ion pumping and subsequent reduction of nerve conduction velocity (NCV) [45, 55, 74]. In addition, augmented cofactor consumption by AR and SDH not only deprives glutathione reductase of NADPH and the capacity to regenerate reduced glutathione (GSH) [45] but also contributes to an imbalanced redox state of NADH/NAD^+ [75], thus promoting oxidative and vascular injury [63, 76, 77].

Overall, the above and numerous other observations obtained from the use of animal models demonstrated consistently that increased polyol metabolism is a strong and readily reversible component in the pathogenesis of diabetes-induced degenerative changes. However, data from human studies indicated no convincing association between the elevation of glucose flux via AR and neuropathic development. Whereas nerves from amputated limbs of diabetic individuals contained significantly higher concentrations of sorbitol and fructose than non-diabetics [78], an assessment of sural nerve biopsies by Dyck *et al.* found that over two thirds of subjects with mild to severe clinical signs or symptoms of DPN had a normal polyol content [79]. A later study by the same group was able to show an inverse relationship between nerve sorbitol level and myelinated fiber density but not other neurological parameters [80]. Importantly, none of the nerve specimen analyses identified a decrease in myo-inositols in relation to DPN, in contrast with the invariable observations of myo-inositol deficiency in rodent models. Likewise, dietary supplementation of myo-inositol prevented and reversed a variety of pathophysiological processes associated with early DPN in rats [81, 82] but failed to normalize any peripheral nerve deterioration in patients with a recent diabetes onset [83, 84]. Nevertheless, the prominent success of AR inhibitors (ARIs) in preventing and reversing experimental diabetic cataract and neuropathy [58, 60, 85-89] as well as the findings of AR gene polymorphisms in diabetic microvascular complication [90-93] spurred a broad enthusiasm in the clinical exploration of these ARIs. While the use of various ARIs almost always prevented or reversed the lens opacification in diabetic rats [94], whether they can reduce the risk of cataract formation in human diabetics remains unclear. This is because most experimentally induced diabetic cataracts occur acutely and possess distinct morphological alterations similar to the features seen with the rare juvenile form of diabetic cataract. Contrasting the juvenile form, the majority of cataracts in diabetes has a dubious sorbitol increase and is represented by the slow, refractive cataract change in diabetic adults [95]. Therefore, a direct evaluation of the use of ARIs as an anti-cataract treatment is difficult in these animal models.

With regard to DPN, two earliest ARIs to be tested for their clinical efficacy in treating DPN were Alrestatin and Sorbinil, which were the prototypic ARIs belonging to the chemical classes of succinimide and spirohydantoins, respectively. Alrestatin produced minor subjective benefit but no improvement on NCV or other objective examinations [96, 97]. While Sorbinil moderately reduced the NCV decline and increased the density of regenerating myelinated fibers in sural nerves [98, 99], its influence on pain and vagal function is questionable and no meaningful therapeutic effects were experienced by patients with diabetic autonomic or polyneuropathy [100-102]. Both Sorbinil and Alrestatin were withdrawn from the clinical setting due to a high rate of toxicity involving photosensitive skin rash [1, 14]. Tolrestat, an acetic acid compound, was able to halt the progression of subclinical peripheral and autonomic deficits in a 52-week duration but had only a mild benefit on chronic symptomatic sensori-motor neuropathy [103-106]. The poor electrophysiological outcome and the incidence of fatal hepatic necrosis eventually led to discontinuation of Tolrestat study [107]. In the cases of the carboxylic acid class of ARIs, Ponalrestat manifested minimal tissue penetration and nerve sorbitol reduction, in spite of its good pharmacokinetics and pharmacodynamics in diabetic rats [108-110]. Although Zopolrestat and Zenarestat demonstrated a dose-dependent amelio-ration in NCV deficits, both of them failed to significantly improve the clinical endpoints

without causing serious adverse reactions [111, 112]. Ranirestat, or As-3201, emerged as a spirosuccinimide with a better drug profile, and was effective in increasing NCVs and sensory function in a phase II trial of mild to moderate diabetic sensorimotor polyneuropathy [113]. The large-scale long-term Phase III trial of Ranirestat, however, did not show statistically significant differences in sensory parameters compared to placebo at all doses tested [114]. Another spirohydantoin, Fidarestat, displayed increased tolerability and a similar degree of improvement in subjective measures to that of Sorbinil [115]. After phase III evaluation, a minor therapeutic value was concluded for Fidarestat in the literatures and its further development was suspended for financial reasons [14, 116]. To date, Epalrestat is the only ARI approved for clinical use in Japan. Despite its success in delaying nerve conduction and sensory abnormalities in a randomized, open label, controlled multicenter trial among Japanese patients [117], the efficacy of Epalrestat has not been confirmed in other populations and appears only marginal in other documentations [1, 118]. In an attempt to identify a meaningful treatment effect of ARIs for clinical DPN, Chalk *et al* conducted a meta-analysis for 13 trials of ARIs involving 879 treated participants and 909 controls. This report found no difference in the overall outcome (SMD -0.25, 95% CI -0.56 to 0.05), nerve conduction parameters or foot ulcers between treatment and control group [119]. Similarly, a previous meta-analysis of studies published before 1996 testing four different ARIs indicated that AR inhibition achieved less than 1 m/s offsets in the decline of median and peroneal motor nerve conduction velocity (MNCV) as the single true statistical change [120]. Given these inconclusive results and safety issues, FDA has not approved any of the aforementioned agents for pharmacological inter-vention of DPN. Although a number of confounding factors, including unexpected placebo effect and trial design, have been blamed for the disappointing clinical outcome, the lack of clear sensory protection by ARIs puts the relevance of polyol pathway to DPN into question.

2.1.2. Advanced glycation and aminoguanidine

Animal and cell studies have well established the contribution of advanced glycation end products (AGEs) to diabetic tissue damage. Nerves, retina and kidney do not depend on insulin for glucose uptake and absorb this energy substrate as a direct function of the circulating glucose concentration. Prolonged hyperglysolia cultivates the glucose autoxidation, decom-position of the Amadori products (fructosamines) following adduction of glucose to the amino groups of lysine residues in the proteins, and fragmentation of glycolytic intermediates (such as glyceraldehyde-3-phosphate and dihydroxyacetone phosphate). All of these gives rise to glyoxal, 3-deoxyglucosone and methylglyoxal within the cells [121]. These highly reducing dicarbonyls are AGE precursors or glycating agents that non-enzymatically react with intracellular nucleotides, proteins, lipids, extracellular matrix and plasma components [122]. The last one is best reflected by the elevated serum glycosylated hemoglobin [HbA1c] level in diabetes. AGE modification of growth factors [123], endocytotic proteins [124], cytoskeletal actin and filaments [125, 126], interstitial matrix and adhesive molecules [127] as well as serum albumin [128] were found in increased amounts in hyperglycemia-treated endothelial cells or diabetic rats and these associated with increased vascular damage, endocytosis, cytoskeletal disassembly, fluid filtration and albuminuria. In both human diabetics and STZ-rats, there was enhanced AGE deposition in peripheral nerves compared to healthy controls as indicated by

immunohistochemical assay [129, 130]. Particularly, pentosidine, a long-lived AGE marker, was significantly elevated in the cytoskeletal protein extracts isolated from diabetic subjects [130, 131]. Moreover, nerve specimens that harvest more AGEs also manifest lower myelinated fiber density.

With respect to intervention, aminoguanidine was the earliest chemical characterized for its anti-glycation activity. It is a hydrazine that preferably and competitively binds to AGE precursors and prevents further irreversible protein glycation [132]. Later studies discovered that besides inhibition of AGE formation, aminoguanidine can negatively act on inducible nitric oxide synthase [133], amine oxidase [134] and reactive oxygen species [135]. Such plethoric pharmacological properties suggest that aminoguanidine is not an appropriate investigational tool for the role of advance glycation in diabetic pathology. However, the continuous use of this compound in preclinical and clinical research was justified by its promising therapeutic effects in rat model of diabetic nephropathy [136], retinopathy [136] and neuropathy [137]. Whereas treating diabetic rats with various doses of aminoguanidine prevented or ameliorated the decrease in nerve blood flow, slowing of NCVs, endoneurial microvessel expansion and failure of sensory nerve regeneration [137-141], subcutaneous injection of aminoguanidine did not improve any of the structural or functional abnormalities in STZ-induced type I diabetic baboons [142]. Although the authors concluded that accumulation of AGEs is not likely an early mechanism of nerve damage in DPN, this discrepancy may also reflect considerable species differences. Indeed, none of the large standardized clinical trials proved a significant advantage of aminoguanidine over placebo in patients, who had well-established diabetic nephropathy [143, 144]. Rather, aminoguanidine adversely affected gastrointestinal, hepatic, respiratory and immune functions and finally led to termination of the studies. For these reasons, no further evaluation of the efficacy of amino-guanidine in treating DPN was pursued.

2.2. Blocking signaling conducers

2.2.1. Protein kinase C and ruboxistaurin

Protein kinase C (PKC) is a ubiquitous serine/threonine kinase of numerous isoforms and cellular functions. Observations in retinal and glomerular tissues from diabetic animals *in vitro* and *in vivo* support the hypothesis that elevated glycolysis subsequent to hyperglycemia dramatically raises 1,2-diacylglycerol (DAG) synthesis. In turn, DAG activates a majority of PKC family members, including PKC-α and -β [45]. Enhanced expression and activity of PKC isoforms, primarily PKC-β, pathologically affect vascular contractility and permeability thereby compromising microcirculation and causing microvascular occlusion [14, 145]. These deleterious consequences have been suggested by many to contribute to the vascular insults and development of retinopathy, nephropathy and cardiovascular disorder in diabetes. However, DAG and PKC upregulation is not a uniform pattern of change in every complication-prone tissue. Unlike the findings in nonneural diabetic complications, nerve DAG levels fall in diabetes and experimental rodent models have presented decreased, increased and unaltered PKC activity [146-148]. Studies of mesangial and smooth muscle cells have linked

PKC activation to diminished Na^+/K^+-ATPase function [149]. On the other hand, both PKC antagonists and agonists normalized Na^+/K^+ pumping in peripheral nerves of diabetic animals, suggesting a conflicting involvement of PKC enhancement and diminishment in the mechanism of Na^+/K^+-ATPase deficits [146]. It is thus intriguing how administration of a PKC-β selective inhibitor, LY333531, restored sciatic nerve blood flow and NCVs in STZ-induced diabetes [150, 151]. In addition, little data from humans, if any, has been obtained to support a PKC change in diabetic peripheral nerves. These experimental results nonetheless implicated PKC inhibition as a prospective avenue for anti-diabetic complication to investigators. The same inhibitor LY333531 (by Eli Lily) with a generic name Ruboxistaurin entered clinical evaluation as a treatment for DPN. In the trial of a small cohort of patients, Vinik *et al* reported that a 32 mg/day Ruboxistaurin for 6 months elicited significant alleviation on skin microvascular blood flow, total sensory symptoms and quality of life [152]. Recently, a 18-week treatment of Ruboxistaurin to a smaller subset of patients with type 2 diabetes proved beneficial in improving total symptom score (NTSS-6) and quality of life [153]. Unfortunately, this did not translate to a multinational, randomized, phase II, double-blind, placebo-controlled study consisting of 205 patients at an equal or double dosage of Ruboxistaurin [154]. Although Ruboxistaurin is well tolerated, Eli Lily withdrew its marketing authorization application.

2.3. Increasing neurotrophic support

2.3.1. Growth factors and growth factor replacement therapy

Mammalian nervous system depends on a group of endogenous and heterogeneous biomolecules for proper physiological functions including growth, survival, differentiation and regeneration. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) from the neurotrophin family are retrogradely transported to neuronal cell bodies after secretion from organs innervated by nerve terminals. These three neurotrophins regulate the activity of small nociceptive and sympathetic sensory fibers, medium size sensory and motor fibers, large diameter sensorimotor and sympathetic neurons, respectively [155]. Other frequently studied growth factors in this context are glial-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF) as well as insulin-like growth factor-1 (IGF-1), which are expressed by peripheral glia and/or neurons and manifest diverse trophic effects on sensory, motor and autonomic nerves [156]. In experimental rodent models, the protein and/or mRNA levels of NGF, BDNF and NT-3 have been observed to both upregulate and downregulate in peripheral nerves, sensory glia and such target tissues as skin keratinocytes, skeletal muscles and submandibular glands [157-164]. Despite these conflicting reports, it is generally believed that the retrograde and anterograde axonal transport of these neurotrophins are diminished in diabetic nerves [14, 165]. Similarly, IGF-1 and CNTF were found to be reduced in various tissues examined in type 1 and type 2 diabetic rat models [166-168]. In STZ or diabetic BB/Wor rats, deficient NGF and IGF-1 level correlated with inadequate macrophage recruitment and Wallerian degeneration after sciatic nerve injury [166, 169]. As postulated by the authors and others, this may explain the perturbed nerve regeneration in diabetes. Considering the highly dynamic nerve degeneration/regeneration in the initial stage

of DPN, growth factor therapy early in disease progression may minimize the damage and aid the axonal repair. To this end, abundant support has been produced using an array of spontaneous, chemical or transgenic diabetic models administered recombinant growth factors. Of note, NGF treatment restored neuropeptide level, C-fiber function and dermal myelinated innervation, alleviated neuropathic pain and promoted injury repair [156]. Whereas BDNF and NT-3 elicited a preferential attenuation in the structural and functional changes of large myelinated sensory and motor fibers [14], GDNF and IGF-1 showed a broad preservation of somatic and autonomic nervous system [19, 156]. Moreover, CNTF adminis-tration prevented/rescued behavioral and electrophysiological dysfunction, and enhanced sensory nerve resprouting in rats previously injected STZ [168, 170].

In humans, there is no prevailing trend of change in the serum level of NGF in type 2 cohorts with symptomatic DPN [171, 172]. Another study revealed significantly weaker immunoreac-tivity of NGF in the lateral calf skin of a group of type 1 diabetics who presented with asymptomatic, early length-dependent loss of nociception and axon reflex vasodilation [173]. However, analysis of the same site from a mixed population of type 1 and type 2 patients with mild early neuropathy indicated that expression of NGF transcripts was higher compared to healthy individuals [174]. Furthermore, epidermal NT-3 protein level markedly increased as a function of the severity of diabetic polyneuropathy [175], whereas CNTF did not vary in postmortem sciatic nerve autopsies between normal and DPN subjects [176]. Likewise, sural nerve IGF-1 mRNA expression was not altered by different durations of DPN [177]. Differing from the findings in animal nerves [161, 178], diabetic humans who developed neuropathy express more trkA and trkC, specific receptors for NGF and NT-3, in the epidermis than those without neuropathy [179]. Whether this reflects a tissue-specific response to diabetes awaits further examination of human nerve biopsies. Clinical testing of recombinant human NGF (rhNGF) perhaps witnessed one of the most spectacular failures in DPN trials. A phase II trial on 250 patients for 6 months reported a robust amelioration on subjective and objective sensory measurements, particularly the components related to small fiber sensory function [180]. When proceeded to a large-scale, multicenter,1-year phase III trial, 1019 participants randomized to receive either placebo or subcutaneous injection of rhNGF could not confirm a neuroprotective effect [181]. Most importantly, severe painful side effects including injection site hyperalgesia and diffuse myalgia significantly limited the tolerable dose to less than 1µg/kg, a dosage 1000 times lower than most of those used in experimental models. This contradicts preclinical data from rodents in which application of NGF reduced pain thresholds [156, 182]. On the opposite side, the observation that NGF evokes pain or hypersensitivity in both animals and humans led to the conception that anti-NGF therapy may reduce neuropathic pain [183-185]. This appeared to be the case in a variety of chronic inflammatory and cancer pain models in which hyperalgesia and/or allodynia were effectively attenuated by antibodies blocking NGF or TrkA [186-188]. In this regard, some proof-of-concept, positive results have been generated in a recent phase III trial on osteoarthritis for a monoclonal antibody against NGF (tanezumab) [189]. However, Pfizer had to temporarily suspend the studies involving DPN after disease worsening and joint replacements occurred in the treatment group.

Other than NGF, a double-blind, placebo-controlled study was also conducted for rhBDNF but found no evidence of improvements on the primary endpoints associated with diabetic sensory neuropathy [190]. Although rhGDNF and rhNT-3 were supposed to enter early clinical assessments for DPN management, they have not yielded any clinical report except the withdrawal of NT-3 from phase I study [156]. It is therefore apparent that the expected outcomes were not met. For IGF-1 and CNTF, development of replacement therapy is also hindered by their non-specific impacts on the central nervous system [191] and muscles [192], respectively.

2.4. Modulating neurovascular function

2.4.1. Nerve blood flow and angiotensin-converting enzyme inhibitors

Multiple epidemiological analyses have previously identified that hypertension strongly increases the occurrence and severity of DPN in population studies [1]. Spontaneously hypertensive diabetic rats developed a more severe behavioral, physiological and structural phenotype pertinent to clinical DPN [193]. Tissues of neuropathic diabetic patients manifest augmented vasoconstrictive response and diminuted endoneurial blood flow [194]. In turn, vascular deficiency and impaired peripheral nerve perfusion contribute to neural hypoxia and ischemia, two of the well-recognized factors in the pathogenesis of microvascular complications in diabetes. This provides a rationale for enhancing vasodilation as a treatment regimen in counteracting diabetes-induced neurovascular stress. This assumption is backed by the observations in experimental diabetes that motor and sensory conduction deficits were normalized by several vasodilating agents with distinct pharmacological actions [195-197]. The most well-established class of compounds in this scenario is angiotensin-converting enzyme (ACE) inhibitors. ACE inhibitors stimulate endothelium-dependent release of nitric oxide and vessel relaxation by antagonizing ACE-mediated formation of the potent vasoconstrictor angiotensin II and deactivation of bradykinin, a strong vasodilator [198]. Combination of these hypotensive effects by ACE inhibitors corrected reductions in nerve blood flow, capillary densities and conduction measurements in STZ-induced diabetic or Zucker fatty rats [199-201].

Although ACE inhibitors are the first line treatment for nephropathy and cardiovascular condition in diabetes [202], there is scarce evidence suggesting the same for diabetic neuropathy. To date, only one small double-blinded, randomized, placebo-controlled DPN clinical study has been conducted on one ACE inhibitor, trandalapril [203]. In this study, normotensive DPN patients treated with trandalapril over 1 year demonstrated significant improvements in electrophysiological function but not QST, neuropathy symptom/deficit score or autonomic function. A major disappointment came from the Appropriate Blood Pressure Control in Diabetes (ABCD) trial. This prospective study followed 470 type 2 diabetic patients for 5.3 years and found neither moderate nor intensive blood pressure control using nisoldipine (Ca^{2+} blocker) or enalapril (ACE inhibitor) was effective in modulating the progression of diabetic triopathy (neuropathy, nephropathy, retinopathy) [204]. Furthermore, there were no overall differential outcomes between interventions. This result along with the fact that clinical

DPN develops and exacerbates in many patients that regularly take the ACE inhibitor casts reasonable doubt on the extent to which ACE intervention is useful in DPN management [205].

2.4.2. Vascular supply and vascular endothelial growth factor therapy

Another approach to address vascular insufficiency is to promote the angiogenesis via expression of vascular endothelial growth factor (VEGF), a cytokine primarily mitogenic for vascular endothelial cells. Overexpression of VEGF through gene transfer stimulated vascularization in both animals [206, 207] and humans [208, 209]. Diabetes was shown to compromise the expression of this growth factor in the skin of patients who also had loss of intraepidermal nerve fiber density (IENFD) [210]. In comparison, most evidence derived from diabetic rodents contradicts with this finding and indicates an upregulation of VEGF in diabetic tissues [211] thatcan be normalized by insulin or NGF infusion [212]. If these observations are true, this could mean VEGF is differentially involved in the pathogenetic processes underlying human and rodent DPN. It is paradoxical, however, that preliminary studies using the same models in which pathological VEGF induction by diabetes was seen also generated data favoring VEGF-enhancing gene therapy in treating DPN. For example, subcutaneous inoculation of herpes simplex virus carrying VEGF-transgene in STZ rats prevented multiple characteristics of experimental DPN, particularly those associated with dorsal sensory function [213]. In a separate report, intramuscular delivery of plasmid DNA encoding VEGF-1 or VEGF-2 completely reversed attenuation of nerve blood flow, slowing of NCV, destruction of vasa nervorum, and dysfunction of small and large fibers in STZ rats [214]. The same study was also able to reproduce the results in rabbits with alloxan-induced diabetes. Two randomized controlled trials (RCTs) have been undertaken to translate this experimental approach to clinical usage. The first trial tested intramuscular VEGF-1 or VEGF-2 gene transfer in 50 DPN patients with presenting symptoms of pain and/or numbness, and achieved an improvement on symptom score, regions of sensory loss and visual analog pain scale over 6-month duration [215]. Other primary and secondary endpoints including quantitative sensory and electrophysiological testing were not met. In addition, there were significantly more severe adverse events in gene therapy group compared to placebo group. Among the listed events, hemorrhage, diabetic retinopathy and peripheral edema had been previously brought up as concerns but apparently were not properly addressed during preclinical animal evaluation [216]. The second trial was reported in a published meeting presentation by Sangamo BioSciences, which announced the phase I/II results for a series of injectable plasmids encoding VEGF genetargeting zinc-finger DNA-binding transcription factor with proven-efficacy in experimental models [217]. Of these, SB-509 was praised to be well-tolerated with a most positive outcome in sensory nerve conduction velocity (SNCV), IENFD and neuropathic impairment score. However, the treatment arm as a whole did not obtain a convincing benefit versus placebo to make this a successful trial. With an argument by Sangamo that a carefully chosen cohort may be more sensitive to SB-509, a latest phase IIb study was set to recruit 170 patients with moderate or severe DPN. Despite broad outcome measures and rigorous analysis, the trial was concluded as being unequivocally disappointing which led to the eventual cessation of this Sangamo's lead program [218].

2.4.3. Lipid metabolism and γ-linolenic acid

γ-Linolenic acid is an important precursor for arachidonic acid. The latter produces the potent vasodilator and platelet inhibitor prostacyclin or prostaglandin I_2 (PGI_2) [219], lack of which can increase the risk of developing thrombosis in diabetic vessels [24] and microvascular diseases. γ-Linolenic acid is primarily synthesized from the dietary ω-6 essential fatty acid linolenic acid, but this reaction is impaired in STZ or alloxan-treated rats [220, 221]. In human type 1 diabetic patients, disturbed fatty acid metabolism has also been inferred from the serum lipid profile [222, 223]. Since γ-linolenic acid also forms the neuronal phospholipids [224, 225], direct supplementation of this polyunsaturated fatty acid can theoretically treat DPN by enhancing both microcirculation and membranous structures in the nervous system, such as the myelin. In keeping with this hypothesis, administration of γ-linolenic acid prevents or reverses the development of experimental DPN in rodents [226-229]. Clinical assessments of the evening primrose oil, the herbal source of γ-linolenic acid, took place in the United Kingdom and suggested an efficacious treatment effect on human DPN [230, 231]. However, some negative outcomes have been obtained for γ-linolenic acid in other clinical conditions by independent groups [232, 233] and the British General Medical Counsel filed a report that the efficacy of evening primrose oil in diabetics claimed by one company-funded trial was falsified [234]. Some issues related to marketing fraud and publication suppression by the drug company attempting to develop evening primrose oil for clinical use have also been raised [235, 236]. Due to these controversies, UK's Medicines Control Agency withdrew the drug's product license. As of today, no further evidence has been acquired to confirm the validity of γ-linolenic acid as an anti-DPN medicine.

2.5. Counteracting oxidative stress

2.5.1. Reactive oxygen species and α-lipoic acid

After years of investigations through experimental approaches which harvested knowledge on a plethora of biochemical pathways linking hyperglycemic stress to nerve injury, a general consensus has been reached by the DPN research community that all these complex molecular and cellular events converge on and interact with one universal consequence, oxidative stress [45, 237]. Direct and indirect evidence of oxidative stress in tissue sites of diabetic complications is overwhelming in animals with induced diabetes. In general, hyperglycemia induces a composite oxidative insult to neurons, SCs as well as vasa nervorum through: 1) accelerated free radicals production; 2) increased oxidation and nitration of proteins, lipids and nucleic acids; and 3) deprivation of antioxidant defense system [238]. Many excellent reviews have illustrated and discussed the pathophysiological consequences of redox imbalance in the peripheral nervous system (PNS) [45, 239] therefore an elaborated description will not be provided here. Briefly, increased intracellular glucose metabolism through the classical glycolytic tricarboxylic acid cycle leads to mitochondrial nutrient overload and subsequently uncontrolled superoxide (O_2^-) production by its oxidative respiratory machinery. Excessive generation of superoxide in conjunction with polyol synthesis exhausts the detoxificating agents including superoxide dismutase and GSH. This eventually gives rise to accumulation

of other reactive oxygen (ROS) and nitrogen species (RNS) such as hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•) and peroxynitrite (NO•). Other hyperglycemia-initiated events such as AGE formation and NGF deficiency have also been suggested to fuel the ROS generation in various compartments. These highly reactive free radicals can non-specifically oxidize and nitrosylate cellular/extracellular biomolecules and undermine organellar function. Particularly, increased protein nitration, lipid peroxidation products and mitochondria dysfunction are predominant phenomena in DRGs and sciatic nerves in diabetic animals [240-242]. Compared to the clear evidence of oxidative damage in experimental DPN, expression of the correspondent biomarkers indicating oxidative stress in human tissues is rather vague [239, 243]. Some studies even suggested a reduced free radical reaction in diabetic patients versus normal control [244, 245]. Further, despite a strong rationale and the promise of substantial neuroprotection by anti-oxidant treatments in rodent diabetics [246-249], this anti-oxidative approach is not spared from the irreproducibility of the results obtained from basic research in clinical practice.

Among a number of anti-oxidants that corrected experimental DPN, α-lipoic acid (ALA) has gone the furthest into clinical use, while the others have proven largely ineffective [14, 250]. ALA or thioctic acid is naturally synthesized in mitochondria and has a powerful antioxidant capacity because of its dual ability to scavenge ROS/transition metals and regenerate other endogenous antioxidants. Approximately 7 double-masked multicenter RCTs, including the series of ALADIN, SYDNEY and NATHAN, testing the efficacy of ALA in treating symptomatic DPN have been completed in Europe [251]. Of these, a general benefit on sensory symptoms and deficits was extrapolated by a meta-analysis incorporating 4 trials (ALADIN I, ALADIN III, SYDNEY, NATHAN II) that treated subjects with 600 mg/day ALA via intravenous infusion for 3 weeks [252]. However, there is an overall mixed bag of results and several therapeutically important indices including symptoms score, nerve conduction and QST were not consistently ameliorated in these studies [205, 252, 253]. Notably, some asserted improvement fell below the clinically meaningful threshold of 30% when adjusted to placebo control [254]. It is also discouraging that trials in which patients received oral dosing of ALA presented only marginal benefit; this significantly precludes the oral application of ALA. Although ALA has been marketed in Germany for treating DPN and is available as nutritional supplement in the US, current existing evidence suggests that ALA at best only retards the neuropathic progression in diabetes.

3. Scientific rationale for the limited translational success: What have we learned?

Based on the records published by National Institute of Neurological Disorders and Stroke (NINDS), a main source of DPN research, about 16,488 projects were funded at the expense of over $8 billion for the fiscal years of 2008 through 2012. Of these projects, an estimated 72,200 animals were used annually to understand basic physiology and disease pathology as well as to evaluate potential drugs [255]. As discussed above, however, the usefulness of these pharmaceutical agents developed through such a pipeline in preventing or reducing neuronal

damage has been equivocal and usually halted at human trials due to toxicity, lack of efficacy or both (Figure 1). Clearly, the pharmacological translation from our decades of experimental modeling to clinical practice with regard to DPN has thus far not even close to satisfactory. Undoubtedly, the flawed design of some clinical trials has led to the inadequate evaluation of certain candidate compounds and for a thorough discussion on this specific topic the readers are referred elsewhere [256]. In this section, we focus on discussing some of the fundamental species differences that render a direct translation unrealistic.

3.1. Failure to predict toxic effects

Whereas a majority of the drugs investigated during preclinical testing executed experimentally desired endpoints without revealing significant toxicity, more than half that entered clinical evaluation for treating DPN were withdrawn as a consequence of moderate to severe adverse events even at a much lower dose. Generally, using other species as surrogates for human population inherently encumbers the accurate prediction of toxic reactions for several reasons.

First of all, it is easy to dismiss drug-induced non-specific effects in animals—especially for laboratory rodents who do not share the same size, anatomy and physical activity with humans. Events such as cardiac attack are often overlooked without a complex and careful examination. A case in point is the anti-diabetic drug Avandia for which the market approval has been a center of dispute. Avandia's active ingredient rosiglitazone promotes insulin sensitivity by activating peroxisome proliferator-activated receptors (PPARs) and was claimed by its maker GlaxoSmithKline to be safe in the preclinical report. Some even went further to advocate the favorable application of rosiglitazone to heart conditions based on its positive influence on cardiovascular biomarkers in rodent studies [257, 258]. Only after accumulating incidents of congestive heart failure among patients receiving Avandia was presented to the FDA, did it begin to spur wide concerns and active investigations of the serious cardiotoxicity by Avandia in humans and animals [259].

Second, some physiological and behavioral phenotypes observable in humans are impossible for animals to express. In this aspect, photosensitive skin rash and pain serve as two good examples of non-translatable side effects. Rodent skin differs from that of humans in that it has a thinner and hairier epidermis and distinct DNA repair abilities [260]. Therefore, most rodent stains used in diabetes modeling provide poor estimates for the probability of cutaneous hypersensitivity reactions to pharmacological treatments [261]. Although skin engraftment onto nude mice has been attempted to circumvent this issue [260], mice with immunodeficiency do not constitute an appropriate background for studying diabetes. Another predicament is to assess pain in rodents. The reason for this is simple: these animals cannot tell us when, where or even whether they are experiencing pain, leaving us to read. Since there is not any specific type of behavior to which painful reaction can be unequivocally associated, this often leads to underestimation of painful side effects during preclinical drug screening (e.g. rhNGF).

The third problem is that animals and humans have different pharmacokinetic and toxicological responses. For instance, troglitazone (Rezulin), another anti-hyperglycemic PPAR agonist,

was withdrawn after inducing idiosyncratic liver failure in patients but a similar hepatotoxicity could not be reproduced in animal models [262, 263]. Even in organ systems that were previously defined as having an overall high rate of interspecies toxicity concordance, unanticipated drug toxicity can still occur. This was the case for trastuzumab (Herceptin), a humanized monoclonal antibody that treats advanced breast carcinoma by binding and blocking human epidermal growth factor receptor 2 (HER2). Both preclinical and on-going toxicological studies in rhesus monkeys and rodents indicated no evidence of cardiac dysfunction [264]. However, trastuzumab administration to patients during clinical trials caused frequent and severe cardiomyopathy [265]. As discussed in a published scientific document of Herceptin toxicity by the European Medicines Agency, it is also unsuitable to assess the cytotoxicity of this antibody that specifically recognizes a single human protein in nonhuman species which have a distinct molecular and immunogenic environment [264]. In addition to the inaccuracies, disparities in pharmacokinetics underpin some of the extreme species differences. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced neurotoxicity is a classic example. MPTP becomes poisonous to dopaminergic neurons once metabolized to MPP^+ by the enzyme monoamine oxidase-B (MAO-B) and elicits permanent Parkinson-like symptoms in human subjects [266]. In sharp contrast, MPTP is barely psychoactive in rats since they produce minimal MPP^+ and only mild damage to mouse brains due to much faster clearance of MPP^+ compared to primates [267]. By the same token, 350 mg of aspirin can be eliminated by half from human circulation in about 3 hours but retained in feline plasma for 37.5 hours, which is essentially lethal to these animals [268]. The argument can be finally strengthened by the work of two independent groups, who compared bioavailability between primates, rodents and dogs for various drugs and both demonstrated that no correlation exists between animal and human data [269]. The matter of drug-induced non-specific effects and uniquely human phenotypes may theoretically be resolved via rigorous pathological evaluation and better experimental method. By comparison, the pharmacokinetic and toxicological data highlights profound interspecies barriers and may not succumb to current technical manipulation. Considering some of the drugs were withdrawn when unexpected toxicological outcomes occur in only 1-2% of the population, relying on laboratory models to predict drug safety certainly puts us in a dilemma with very little medical and ethical risks from which our society can suffer (Figure 1).

3.2. Failure to recapitulate human neuropathologies

Genetic or chemical-induced diabetic rats or mice have been a major tool for preclinical pharmacological evaluation of potential DPN treatments. Yet, they do not faithfully reproduce many neuropathological manifestations in human diabetics. The difficulty of such begins with the fact that it is not possible to obtain in rodents a qualitative and quantitative expression of the clinical symptoms that are frequently presented in neuropathic diabetic patients, including spontaneous pain of different characteristics (e.g. prickling, tingling, burning, squeezing), paresthesia and numbness. As symptomatic changes constitute an important parameter of therapeutic outcome, this may well underlie the failure of some aforementioned drugs in clinical trials despite their good performance in experimental tests measuring behavioral responses of animals to external stimuli (Table 1). Development of nerve dysfunction in diabetic rodents also does not follow the common natural history of human DPN. As

described earlier, sensory neuropathy in humans typically adopts a length-dependent, "stocking-glove" loss of sensation that slowly progresses from distal to proximal. Such a pattern was never functionally recapitulated in the commonly used type 1 and type 2 diabetic animal models, including STZ-injected rats, Zucker diabetic fatty (ZDF) rats and db/db mice. Besides the lack of anatomical resemblance, the changes in disease severity are often missing in these models. For example, although the majority of diabetic rodent models developed thermal hypoalgesia with long durations of diabetes as revealed by the sensory assay correspondent to that of QSTs in humans, there is no agreement between different studies in a consistent trend of progressive decline in thermal pain perception [270-272], a well-known phenomenon in patients. Alterations in thermal sensation in the tails of diabetic rodents varied upon studies and species used [273-275] and several groups have documented increased temperature perception after prolonged diabetes [276, 277], thus falsifying the relevance of tail flick test to human conditions. More importantly, foot ulcers that occur as a late complication to 15% of all individuals with diabetes [14] do not spontaneously develop in hyperglycemic rodents. Superimposed injury by experimental procedure in the foot pads of diabetic rats or mice may lend certain insight in the impaired wound healing in diabetes [278] but is not reflective of the chronic, accumulating pathological changes in diabetic feet of human counterparts. Another salient feature of human DPN that has not been described in animals is the predominant sensory and autonomic nerve damage versus minimal involvement of motor fibers [279]. This should elicit particular caution as the selective susceptibility is critical to our true understanding of the etiopathogenesis underlying distal sensorimotor polyneuropathy in diabetes. In addition to the lack of specificity, most animal models studied only cover a narrow spectrum of clinical DPN and have not successfully duplicated syndromes including proximal motor neuropathy and focal lesions [279].

Morphologically, fiber atrophy and axonal loss exist in STZ-rats and other diabetic rodents but are much milder compared to the marked degeneration and loss of myelinated and unmyelinated nerves readily observed in human specimens [280]. Of significant note, rodents are notoriously resistant to developing some of the histological hallmarks seen in diabetic patients, such as segmental and paranodal demyelination [44]. There are sporadic reports of demyelination in STZ and genetically diabetic Bio-Breeding (BB) rats after 8-12 months of diabetes [58, 281-283]. However, this is apparently related to a different microvascular pathology as morphometric analysis of sural and tibial vasa nervorum in these rats revealed dilated lumina, flattening of endothelial cells and microvessel walls [284], contrasting with the basement membrane thickening, endothelial hyperplagia and narrowing of endoneurial lumen in human diabetics [285, 286]. Similarly, the simultaneous presence of degenerating and regenerating fibers that is characteristic of early DPN has not been clearly demonstrated in these animals [44]. Since such dynamic nerve degeneration/regeneration signifies an active state of nerve repair and is most likely to be amenable to therapeutic intervention, absence of this property makes rodent models a poor tool in both deciphering disease pathogenesis and designing treatment approaches. Given that our ability to devise a cure for human DPN depends ultimately on our successful understanding and reduction of its various functional and structural indexes, failure of most animal models to replicate these human neuropathologies with high fidelity renders this task difficult at best.

Species/ Models Characteristics	Humans	Induced	Spontaneous					Transgenic/ Knockout mice
		STZ-rats/mice	BB/Wor-rats	NOD mice	ZDF rats	ob/ob mice	db/db mice	
Disease Genesis	multigenic	monogenic						
Onset	chronic (years to decades)	acute (3 days)	moderate (2-5 months)	unpredictable (4-30 weeks)	mild (1-2 months)			various, superimposed by other diabetogenic protocols
Progression	slow, chronic (years to decades)	Rapid (6-20 weeks)				Slow (11-68 weeks)		
Glycemic Profile	moderate hyperglycemia	severe hyperglycemia	severe/fatal hyperglycemia, requires insulin for life preservation		severe hyperglycemia	normo- to mild hyper-glycemia	moderate hyperglycemia	
Symptoms	varying degree and properties of pain, paresthesia, numbness, insensitivity, absent reflexes, muscle weakness	no clear definition/presentation of spontaneous pain or other symptoms; thermal/mechanical hyperalgesia and tactile allodynia are often used as indication of increased pain perception however cannot be differentiated from increased sensory function						
Sensory Function	thermal hypoalgesia, decreased vibration perception threshold, loss of Achilles reflex	mechanical hyperalgesia, thermal hyperalgesia/hypoalgesia, mechanical/tactile allodynia	thermal hyperalgesia	thermal hyperalgesia/ hypoalgesia, tactile allodynia	thermal/ mechanical hyperalgesia	thermal/ mechanical hypoalgesia	thermal hypoalgesia	various
Nerve Conduction	progressive decrease at a rate of 0.5m/s per year	>10m/s reduction within 6-20 weeks						
Morphology	loss of unmyelinated and myelinated fibers, evidence of axonal degeneration and regeneration, segmental and paranodal demyelination, distal axonotrophy; basement membrane thickening, narrowing of endoneuriallumina	distal axonotrophy, myelinated fiber atrophy, few axonal loss, demyelination only after long-term hyperglycemia; basement membrane flattening, widening of microvascularlumina		axonal atrophy and degeneration	not characterized	slight fiber atrophy and loss; basement membrane thickening, narrowing of endoneuriallumina		various
Nerve Biochemistry	sorbitol, fructose content not altered or slightly elevated, much lower compared to rodents at both healthy and diabetic states; myo-inositol level unchanged	sorbitol, fructose level highly increased by diabetes; myo-inositol level markedly reduced		unclear		sorbitol, fructose level normal or moderately elevated; myo-inositol level unchanged		AR-overexpressing mice exhibit exaggerated increase of polyol pathway metabolites and reduction of myo-inositol
Overall Limitation of the Model		phenotypic exaggeration and acceleration driven mostly by severe hyperglycemia				representing early or prediabetesrather than overt diabetes		amplifying specific components, applicable to rare cases

Table 1. Comparison of DPN Characteristics between Humans and Frequently Used Laboratory Rodent Models

Abbreviations: NOD=non-obese diabetic, AR=aldose reductase

3.3. Overrepresentation of pathogenetic pathways

STZ is a glucose analog of selective toxicity to pancreatic β-cells and induces insulin-deficiency and hyperglycemia mimicking that in human type 1 diabetes mellitus. Injection of this chemical provides a convenient and affordable tool in inducing robust hyperglycemia in animals with good control over disease onset and duration. Therefore, STZ-rats have been favored by researchers during preclinical drug assessments for diabetic complications [280]. However, STZ typically produces a rather immediate, severe hypoinsulinemia and elevation of blood glucose, whereas the development of hyperglycemia in most human conditions is slow and modest [287]. The contrariety manifests stably in the serum HbA1c levels. While the non-diabetic range (~4-5.6%) is similar, a single administration of STZ to Wistar rats can increase the HbA1c to above 12% in 4-5 weeks [288, 289], which indicates a very poor glucose control that is considered rare in the clinic setting with anti-diabetic care. In fact, less than 15% of patients may have an HbA1c level exceeding 9% by sample estimation [290]. Such extreme hyperglycemia in STZ-treated rats could give rise to exaggerated glucose accumulation and metabolic derangements that would not be commonly present in human diabetics. Indeed, the concentrations of sorbitol and fructose per unit weight of nerve tissue in STZ diabetic rats is consistently increased and dramatically higher in comparison with human diabetics, who on average also do not uniformly show upregulation of these glucose metabolites via polyol pathway [44, 55, 79]. Of interesting note, under normal physiological conditions the contents of nerve sorbitol in rodents are almost 10-fold higher than those in humans, suggesting some species difference in the relative involvement of AR in glucose metabolism during both normo- and hyperglycemia. Observations of polyol pathway utilization in different species and cell types vary widely; the total glucose utilization through polyol pathway is one third in rabbit ocular lenses and only one tenth in human erythrocytes in response to high glucose stress [45, 291]. Consistent with an inverse association between increased polyol flux and electrophysiological dysfunction, diabetic rodents frequently exhibit 10 m/s or more reduction in NCV within the typical 6-20 week experimental duration [271, 292-294]. By contrast, the deterioration of NCV in human patients gradually takes place and has an average loss of 0.5 m/s per year [1] (Table 1). It is also suspicious that the profound and precipitated NCV deceleration in STZ-rodents occur without apparent histopathological changes, which can be a prominent feature in diabetic neuropathic patients at early stage. Therefore, enhanced AR activity might contribute differently or less significantly to the pathogenesis of DPN in humans than rodents. This could explain why AR inhibitors, and by extension, many other pathogenetically targeted inhibitors afford potent neuroprotection in experimental studies but only marginal effects in clinical trials.

Another criticism is that most STZ models were rendered diabetic at puberty since administering STZ to rodents after sexual maturation cannot always produce peripheral nerve abnormalities [280, 295]. Unlike matured nerves that displayed little change in response to diabetic insults, immature peripheral nerves readily manifest hyperglycemia-induced morphological and electrophysiological deficits within an even shorter duration [295]. However, such a phenotype bears little relevance to 90% of clinical conditions, in which diabetes-induced nerve damage has an adult onset and slow time course.

3.4. Other physical and environmental factors

Humans certainly share considerable biological similarities with other mammals. In the nervous system, these include some of the nociceptive responses and higher cognitive activities. At the same time, no one would suggest that humans and animals are the same—they obviously differ in many physiological and behavioral aspects. The question is: can we obtain effective therapeutic applicability after evolution has well separated our species from others? In order to answer this, it is necessary to carefully examine these differences and their impacts on the pharmacokinetic and pharmacological extrapolation. As delineating every single molecular, cellular and phenotypic difference is a laborious task, we will highlight only those relevant to our discussion of DPN. When comparing humans with the conventionally used experimental animals, namely rats and mice, the most conspicuous difference is anatomical. With particular respect to neuroanatomy, a peripheral axon in humans can reach as long as one meter [296] whereas the maximal length of the axons innervating the hind limb is five centimeters in mice and twelve centimeters in rats. This short length makes it impossible to study in rodents the prominent length dependency and dying-back feature of peripheral nerve dysfunction that characterizes human DPN. Even if size were an issue and macro-structure appears similar, there might still be striking differences in the micro-structure within the tissue or organ. This is the case for insulin-secreting islets. For decades the cytoarchitecture of human islets was assumed to be just like those in rodents with a clear anatomical subdivision of β-cells and other cell types. By using confocal microscopy and multi-fluorescent labeling, it was finally uncovered that human islets have not only a substantially lower percentage of β-cell population, but also a mixed—rather than compartmentalized—organization of the different cell types [297]. This cellular arrangement was demonstrated to directly alter the functional performance of human islets as opposed to rodent islets. Although it is not known whether such profound disparities in cell composition and association also exist in the PNS, it might as well be anticipated considering the many sophisticated sensory and motor activities that are unique to humans.

Considerable species difference also manifest at a molecular level. The chemical structure and signaling profile of a molecule may not always be conserved throughout the evolution. Such difference, although small, can account for a significant translational limitation for pharmacological treatments targeted at a specific biomolecule. A good explanation is the case of trastuzumab. As mentioned earlier, trastuzumab was specifically designed to immuno-antagonize HER2, thereby inhibiting cancer cell growth. However, this drug could not be adequately assessed in rodents or primates because of the inability of this human protein-targeting antibody to recognize the HER2 homologues expressed in these nonhuman species [264]. Despite the successful employment of nude mice for the preclinical evaluation of trastuzumab, a comprehensive pharmacological and pharmacokinetic profile was not obtained for this humanized antibody and it resulted in unpredicted toxicity in patients. While the molecular difference might not be as serious of a problem for rhNGF and rhVEGF, critical retrospective examination into this aspect may lend some insight into the failure of these gene therapies in DPN trials. At least 80% of human genes have a counterpart in the mouse and rat genome. However, temporal and spatial expression of these genes can vary remarkably

between humans and rodents, in terms of both extent and isoform specificity. The first is evident from the differential level of MAO-B expression in humans and rats which resulted in distinct susceptibility of these two species to MPTP-induced neurotoxicity [266]. The second category involves protein families comprising multiple isoforms owing to different promoter usage and alternative gene splicing. For instance, the enzyme PKC has at least 12 different subtypes, of which, PKC-α is predominantly expressed in human hearts and PKC-ε in rodents [298]. Since activation of PKC-α and PKC-ε are differentially regulated, species-specific PKC inhibitors will need to be developed in order to efficiently block the pathogenic action of this kinase in cardiomyopathy, especially when a non-selective inhibition of PKC function is unwanted or even detrimental. Given that the efficacy of ruboxistaurin in treating DPN was also based on data from rat diabetic models [150, 151], it is imperative to speculate that the unsatisfactory results of ruboxistaurin in patients is due at least in part to a relatively less important role of PKC-β in the pathological development of diabetic human nerves. The last type of molecular difference is that the components along a particular signaling axis may be preferentially vulnerable to pathological alteration in different species. This possibility has been largely ignored but could underpin a major limitation in current translational research. One typical example is that much has been learned regarding the anti-hyperphagic effects of leptin from ob/ob mice, which also led to the exciting finding that administration of this hormone can successfully suppress weight gain [299]. Nonetheless, this offered little treatment benefit for the majority of obese people (99.95%) who have impaired signaling downstream of leptin instead of leptin deficiency as observed in ob/ob mice [300]. Some may argue that these issues can be overcome by creating genetically engineered or "humanized" mice in which a mouse gene is substituted by the human version. However, transgenic or knockout mice can be afflicted with developmental deficits and alterations which are inappropriate for modeling a chronic disease that appears in the later life time, such as type 2 diabetes and its complications. Moreover, we do not know whether a genetically introduced human protein—if it is different enough from the murine orthologue that a transgene is necessary—faithfully maintains the same expression and interaction properties in mouse system as it would in humans.

Ultimately, a fundamental problem associated with resorting to rodents in DPN research is to study a human disorder that takes decades to develop and progress in organisms with a maximum lifespan of 2-3 years. The longest duration of experimental diabetes in a rodent model was documented by Ras *et al.*, who observed leptin-deficient db/db mice for 17 months and reported only mild pathological changes in the peripheral nerve fibers [301]. It is thus fair to say that a full clinical spectrum of the maturity-onset DPN likely requires a length of time exceeding the longevity of rodents to present and diabetic rodent models at best only help illustrate the very early aspects of the entire disease syndrome. Since none of the early pathogenetic pathways revealed in diabetic rodents will contribute to DPN in a quantitatively and temporally uniform fashion throughout the prolonged natural history of this disease, it is not surprising that a handful of inhibitors developed against these processes have not benefited patients with relatively long-standing neuropathy. As a matter of fact, any agents targeting single biochemical insults would be too little too late to treat a chronic neurological disorder with established nerve damage and pathogenetic heterogeneity (Figure 2). In DPN, such heterogeneity is the consequence of a complex interplay between genetic predisposition,

physical characteristics, nutritional and other environmental factors. On the contrary, experimental rodents are maintained at a homogeneous genetic background. Genetic homogeneity becomes particularly apparent with the inbred strains and genetically engineered mice, making them more of a tool to elucidate the contribution of a specific component to disease development and less of a tool for an accurate prediction of the likelihood that a treatment will be effective for a general population. Apart from these internal factors, laboratory caged animals have an uniform dietary constitution, life cycle and environmental contact, therefore would not be exposed to the majority of the external risk factors otherwise incurred by individual patients, such as smoking and alcohol consumption [10]. Finally, humans have some unique behaviors that assume an integral part of DPN-associated complications but cannot be adopted by animals. This is perhaps the simplest reason why diabetic rodents are immune to gangrenous foot ulceration as upright walking has not evolved in these species.
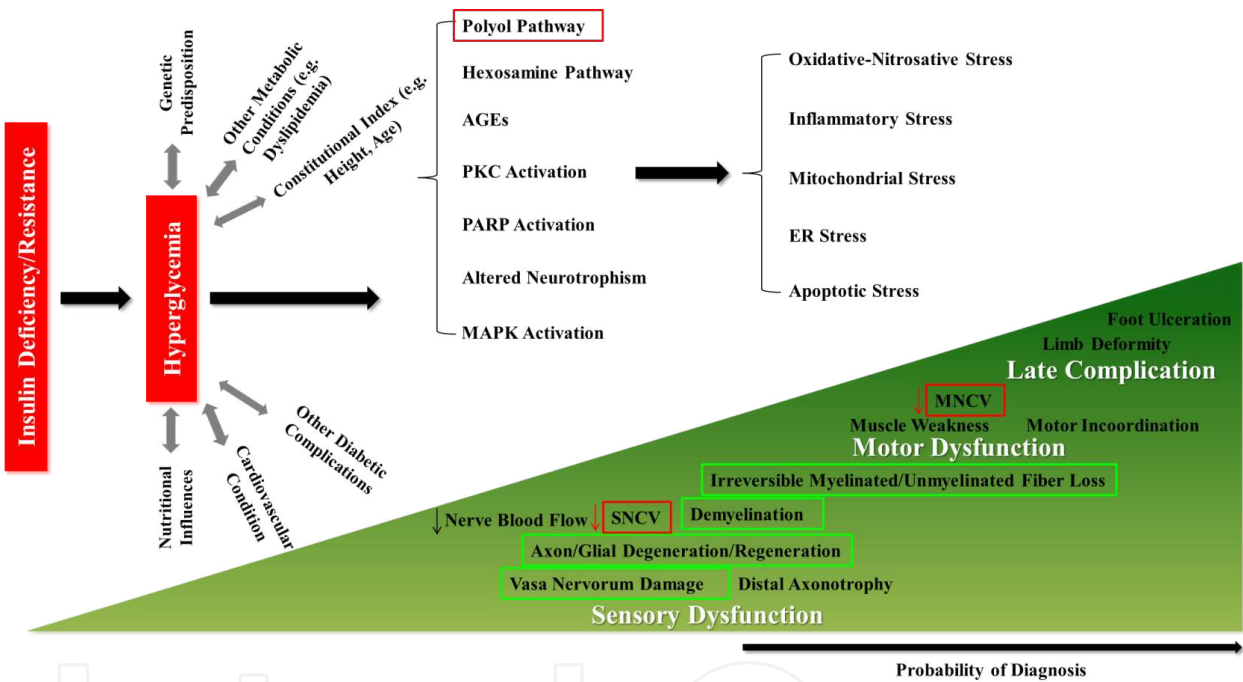

Figure 2. Schematic Demonstration of the Progressive Pathogenetic and Pathophysiological Changes in DPN. Components highlighted in red marks changes that are often over-exaggerated in frequently used rodent models, whereas those in green mark physiological and morphological changes not replicated or misreplicated. Darker color in the triangle box indicates less likely the pathologies are to be adequately modeled in rodents. Double-headed arrows indicate interaction. PARP: poly(ADP-ribose) polymerase, MAPK: mitogen-activated protein kinase, ER: endoplastic reticulum.

4. Conclusion and outlook

Needless to say, DPN has been a significant source of diabetes-induced mortality and morbidity that strike individuals, families and society with a staggering health and economic cost. There is little doubt that the need for effective DPN management is currently unmet and better

therapeutic regimens ought to be sought. The invasive nature of present methods of biochemical, structural and functional measurements dictates that systemic and longitudinal assessments are not feasible in humans. To address this, miscellaneous rodent models have been created and used as substitutes for diabetic patients for the purpose of uncovering the pathogenetic mechanisms and testing potential pharmacological treatments. However, these conventional approaches have so far failed to yield a successful therapeutic translation. Further, animal surrogates are afflicted with species differences in genotype and behavior, nerve structure and metabolism, duration of diabetes, and tissue vulnerability, which allow limited transferability of animal results into clinical settings. It is important to point out that the present review does not argue against the ability of animal models to shed light on basic molecular, cellular and physiological processes that are shared among species. Undoubtedly, animal models of diabetes have provided abundant insights into the disease biology of DPN. Nevertheless, the lack of any meaningful advance in identifying a promising pharmacological target necessitates a reexamination of the validity of current DPN models as well as to offer a plausible alternative methodology to scientific approaches and disease intervention. After a critical reevaluation of the experimental results and clinical outcomes for several previously high-profile anti-DPN drugs, we conclude that the fundamental species differences have led to misinterpretation of rodent data and overall failure of pharmacological investment. As more is being learned, it is becoming prevailing that DPN is a chronic, heterogeneous disease unlikely to benefit from targeting specific and early pathogenetic components revealed by animal studies. Rather, an efficacious therapy must impact on multiple etiologic events and manage various risk factors. In this regard, rigorous lifestyle modulation may simultaneously intervene with a multitude of internal and external diabetogenic processes without generating significant tissue toxicity and side effects. Particularly, diet and exercise intervention provides an approach to improve metabolic management and enhance long-term reparative and regenerative capacity of diabetic nerves. Moreover, investigating the disease process via human-based study to the extent possible promises to lend much better insight into the pathology and pathogenesis of DPN as well as the clinical utility of potential treatments. We propose that future research should put an emphasis on advancing methodological and technological approaches that maximizes the access and utilization of human specimens under ethical guidelines, and on refining lifestyles for preventing and modifying DPN, which are more cost-effective and directly applicable to clinical practice in this otherwise largely intractable disorder.

Acknowledgements

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