Diabetic neuropathy in animal models

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Diabetic neuropathy is not a single but several different pathogenic mechanisms leading to several symptoms. Diabetic animal models have been extensively used to characterize these mechanisms to evaluate potential treatments in humans. We reviewed the chronology of the pathogenic process underlying diabetic neuropathy occurring in several in vivo diabetic animal models in either type 1 or type 2.

Introduction

Neuropathy is the major reason for morbidity and mortality among diabetic patients. Identification of underlying mechanisms is of greatest importance to better understand the failures with existing treatments and develop new approaches for diagnosis and therapy of neuropathies.

Diabetic rodents exhibit many disorders seen in patients with neuropathy including allodynia, nerve conduction slowing and progressive sensory loss [1,2]. However, no one would suggest that diabetic animals faithfully develop human diabetic complications. One of the problems derives from the longevity of rat models in relation to the slow time course of the neuropathy development in patients. Instead of modeling the entire disease syndrome, specific components are also studied such as the involvement of insulin, glucose as well as polyol pathway and axonal neurofilaments on neuropathy development. This review highlights in vivo models that provide the greatest insight into the pathophysiology of diabetic neuropathy.

In vivo animal models

In vivo animal models for diabetic neuropathy studies can be subdivided into two groups: induced models and genetic models that mimic either type 1 (insulin dependent) or type 2 (insulin resistant) diabetes. In addition, other tissue-specific transgenic models can be rendered diabetic to study a specific pathway that might be involved in the pathogenesis.

Diabetic neuropathy in type 1 diabetic models

Streptozotocin (STZ)

STZ is highly toxic for β-cells and is widely used to create rodent models of type 1 diabetes [3]. The development of STZ-induced diabetic neuropathy is mainly dependent on the level and duration of hyperglycemia.

In the early course of diabetes in STZ rats, endoneurial blood flow and micro- and macrovascular reactivity were impaired [1,2]. Slowing of sensory nerve conduction velocity (SNCV) and motor nerve conduction velocity (MNCV), hyperalgesia and allodynia were shown to develop within the first month of the onset of hyperglycemia in STZ rats. After 8–12 months of diabetes, signs of axonopathy, demyelination, nerve degeneration and hypoalgesia can also be detected in STZ rats [1,2].

STZ-diabetic mice have been suggested to be a better model of diabetes than STZ rats, because they have an earlier maturity with a negligible weight loss following diabetes initiation.

Similar to rats, 1-week STZ mice develop vascular dysfunction with no NCV alterations [4,5]. Changes in spinal terminals of calcitonin gene-related peptide (CGRP) in unmyelinated sensory neurons were detectable 4 weeks after diabetes and progressively worsened with time (6–7 weeks) in STZ mice [6].
With increasing duration of diabetes (7–9 weeks), there is a loss in cutaneous C-fiber innervation [7] and decrease in MNCV, SNCV as well as hypalgesia [8].

Nine-month-old diabetic mice showed further reduced MNCV, decreased amplitudes of M waves and nerve action potentials, axon atrophy, myelin thinning as well as loss of epidermal axons, sweat gland innervation and sensory neuron [9].

Antioxidants, aldose reductase inhibitors (ARIs), aminoguanidine, insulin, dietary myo-inositol supplementation and neotrofin alleviated thermal hypoalgesia and NCV deficits in STZ rats [1,2,10]. Antioxidant, ARIs, poly(ADP-ribose)polymerase (PARP) inhibitors and glial cell line-derived neurotrophic factor (GDNF) reversed neuropathy in short- and long-term STZ mice [5,6,8].

Bio-breeding/Worcester rat: BB/Wor-rat

The pancreatic islets of the BB/Wor-rats are subjected to an immune attack leading to insulinitis [3]. In type 1 diabetes-prone strains, hyperglycemia and insulopenia develop at around 12 weeks of age, often at the age of puberty.

The type 1 BB/Wor-rat shows early abnormalities such as an activation of the polyol pathway, with a consequent impairment of neural Na+/K+-ATPase. Endoneurial hypoxemia secondary to impaired endoneurial blood flow contributes to impaired nerve perfusion and further to nerve dysfunction. In the BB/Wor-rats, there is a greater decrease in MNCV than SNCV after 5-week duration of diabetes [11]. One of the earliest detectable changes in myelinated fibers is nodal and paranodal axonal swelling that are associated to other abnormalities of the cytoskeletal structure leading to pertubated axonal transport and progressive axonal atrophy. Significant fiber loss is already detectable in sural nerves of BB/Wor-rats after 4 months of diabetes and increases after 11 months [11]. Thermal hyperalgesia appears progressively up to 6-month duration of diabetes showing damage to small myelinated Aδ and unmyelinated C-fibers and then increases after 8 months showing profound C-fiber loss. This is followed by axonal atrophy and loss, preceded by an impairment of neurotrophic support and synthesis of nociceptive neuropeptides, such as substance P (SP) and CGRP [11]. In addition, in BB/Wor-rats, the development of sympathetic autonomic neuropathy is characterized by neuroaxonal dystrophic changes of terminal axons [12].

C-peptide treatment rapidly corrected endoneurial blood flow, NCV slowing [2,11], thermal hyperalgesia, atrophy and degeneration of C-fibers in type 1 BB/Wor-rats [2].

Despite the intensive study of the immunopathogenesis of islet cell destruction in the NOD mouse, very little work has been done to study complications of diabetes in this model. In part, this is because of the late and somewhat unpredictable age at the onset of diabetes and the requirement for more intensive management, including daily administration of exogenous insulin. In addition, the genetics of the NOD model is complex.

There have been relatively few studies of neuropathy in the NOD line; nonetheless, those few studies indicate that tail-flick latency was unaltered by 2 weeks of diabetes in 12-week-old NOD mice [13] and thermal hypoalgesia was observed in 18-week-old NOD mice [14]. Another study reported that diabetic NOD mice developed a significant time-dependent hyperalgesia that did not correlate with the increase in the plasma glucose concentration, but rather appeared very early alongside diabetes and was significant at young age (8–10 weeks), and lasted up to 32 weeks old [15]. An enhanced neuropeptide Y (NPY) content may increase sympathetic nerve activity and vascular resistance in 12-week-old female non-obese early diabetic animals [16], which clearly manifests early diabetic autonomic neuropathy (23–25 weeks old) [17]. Peroxynitrite inhibitor prevented the reduction in MNCV and hind-limb digital SNCV deficits, thermal hypoalgesia, tactile allodynia and the loss of intraepidermal nerve fibers [14].

**Diabetic neuropathy in type 2 diabetic models**

**Zucker diabetic fatty (ZDF) rat**

ZDF rats are developed from obese male Zucker rats that become diabetic and are selectively bred to create a stable new strain. The onset of diabetes in this strain appears to be related to the loss of GLUT-2 glucose transporters in pancreatic β cells and to concomitant loss of muscle GLUT-4 transporters. Diabetes in this strain is associated with impaired insulin secretion and peripheral glucose transporter function.

MNCV and SNCV were reduced in hyperglycemic 8-week-old male ZDF rats compared with nondiabetic rats [18] and MNCV remained reduced until 40 weeks old [2,19,20].

Acetylcholine-mediated vascular relaxation of epineurial arterioles of the sciatic nerve was impaired from 8 to 10 weeks of age in ZDF rats [19,20]. Sciatic endoneurial nutritive blood flow and sciatic endoneurial vascular conductance were decreased and dorsal root ganglion (DRG) neuron CGRP content was found to be reduced in 14-week-old ZDF rats [18]. Another study reported that sciatic endoneurial blood flow was reduced from 24 to 28-week-old ZDF rats [20]. Sciatic nerve sorbitol concentration was greatly increased in 17-week-old ZDF rats [2].

In 8-week-old ZDF rats, thermal hyperalgesia was observed and was persistent 6 weeks later. By contrast, mechanical hyperalgesia became evident in 10-week-old ZDF rats and...
subsequent time points [18]. At 40 weeks old, CGRP level was severely reduced in epineurial arterioles from ZDF rats [20].

The antioxidant taurine and ARI reverse neurological and neurovascular deficits in ZDF rats (deficits of MNCV, SNCV, nerve blood flow (NBF), thermal and mechanical hyperalgesia and DRG neuron CGRP) [2,18].

Bio-breeding Zucker/Worcester rats: BBZDR/Wor-rat

The inbred bio-breeding Zucker diabetic rat strain is a relatively new and emerging model of type 2 diabetes [3]. Similar to patients with clinical type 2 diabetes, the BBZDR/Wor-rat develops complications associated with hyperglycemia.

In the BBZDR/Wor-rats there is a milder defect in MNCV at 5-week duration of diabetes with no alteration of SNCV compared to the type 1 BB/Wor-rats under the same duration [11]. NCV starts to be decreased only after 8-month duration of diabetes [11]. BBZDR/Wor-rats exhibit milder axonal atrophy and fiber loss than type 1 BB/Wor-rats, amounting to 11% after 14-month duration of diabetes. In BBZDR/Wor-rats, there is an almost normal expression of neurotrophic factors in DRG cells and a milder suppression of nociceptive neuropeptides than in type 1 BB/Wor-rats. BBZDR/Wor-rats do not develop sympathetic autonomic neuropathy [12].

Goto Kakizaki (GK) rat

GK rats were developed by the selective breeding of Wistar rats with the highest blood glucose over many generations leading to insulin resistance and impaired insulin secretion [3].

GK rats aged from 2 to 9 months had a modest hyperglycemia with an impaired glucose tolerance test and exhibited a reduced MNCV [21–23] with a higher incidence of fibers with paranodal, segmental demyelination and axonal degeneration in 9-month-old GK rats [21]. The mean axonal size of unmyelinated nerve fibers was significantly reduced in 2-month-old GK rats and remained reduced until they were 6 months old. By contrast, mean values of nerve fiber size, axonal size and axon/myelin ration were reduced at 6 months old [22].

Nerve sorbitol levels were increased in ~2- and ~5-month-old GK rats leading to a reduced Na+-K-ATPase activity at 9 months old. Nerve myo-inositol levels in GK rats were lower at 9 months old than those of normal controls [23].

It has been reported that there are progressive neuropathic changes with increasing age in GK rats [21–23]. There was a thermal hyperalgesia following long-term hyperglycemia in 9-month-old male GK rats [24]. By contrast, there was no difference in tail-flick latency between 9-month-old female GK rats and control rats [24].

Eighteen-month-old GK rats with impaired glucose tolerance test and insulinopenia had a reduced MNCV associated with a loss of small myelinated fibers, atrophy and loss of unmyelinated axons [25]. Nociceptive fibers impairment was supported by significant decreases in the expression of SP and CGRP in DRG; positive neurons and sciatic nerve further reflecting degeneration of SP and CGRP fibers.

A chronic administration of an inhibitor of Na+-glucose cotransporter (T-1095) lowered blood glucose and glycated hemoglobin levels, partially improved glucose intolerance and insulin resistance, and prevented the development of diabetic neuropathy in GK rats [24].

Lowering high blood glucose levels by the use of α-glucosidase inhibitor for 3 months improved MNCV and nerve demyelination in 9-month-old GK rats [21].

Otsuka Long-Evans Tokushima fatty (OLETF) rat

OLETF rats are derived from spontaneously diabetic Long-Evans rats and characterized by mild obesity and late-onset hyperglycemia (18 weeks old) and impaired insulin secretion at 40 weeks old [3]. Because of the chronic progressive form of diabetes, OLETF rats are suitable for studying the pathophysiological changes during the prediabetic phase.

OLETF rats showed thermal hyperalgesia and disturbed responses to thermal pain at 4 months old [26]. At 9–10 months old, no significant changes were observed in MNCV and sciatic NBF [27], whereas in another study MNCV and thermal nociception started to decrease [28]. 9–10-month-old OLETF rats fed with 30% sucrose developed a diabetic neuropathy that was more severe than OLETF rats [27,28]. Sucrose administration for 8 weeks in OLETF rats caused deterioration of both somatic nerve function, MNCV and autonomic nerve function, which was concomitant with severe hyperglycemia. In addition, these neural dysfunctions were accompanied by reduced sciatic NBF, hyperactivity of the polyol pathway, resulting in sorbitol and fructose accumulation and myo-inositol depletion [27].

ARI treatment can reverse a severe diabetic neuropathy developed in 9–10-month-old OLETF rats fed for 8 or 24 weeks with 30% sucrose [27].

ob/ob mouse

Mice that are ob/ob have no leptin action (genetic mutations in leptin) and are obese, insulin-resistant, hypertriglyceridemic, but normoglycemic [3].

In 11-week-old ob/ob mice, a recently characterized model of neuropathy associated with type 2 diabetes and obesity, large motor fiber neuropathy (MNCV deficit), large sensory fiber neuropathy (digital SNCV deficit) as well as small sensory fiber neuropathy (thermal hypoalgesia, tactile allodynia) have been reported [29].

They also had increased nitrotyrosine and PARP immunofluorescence in the sciatic nerve, spinal cord and DRG neurons. Moreover, a severe reduction in intraepidermal nerve fiber density (INFD) in ob/ob mice exceeded that observed in STZ-diabetic mice with 8–12-week duration of diabetes that had more severe hyperglycemia, but no obesity [29].
Six weeks of ARI administration in 11-week-old ob/ob mice prevented MNCV and SNCV deficits, thermal allgesia, INFD loss but not tactile allodynia [29].

db/db mouse
db/db mice have a defective binding of leptin, leading to obesity, insulin resistance, hyperglycemia and hypertriglyceridemia [3].

In db/db mice younger than 1 month old, MNCV is not altered, but with longer durations of diabetes, deficit develops [1,30]. The average area density of nerve in the epidermis is significantly reduced in db/db mice [31,32], well correlated with previous observations that diabetic patients had reduced numbers of cutaneous nerves [31]. At 6 months of age, morphometric examination of sensory and motor nerves at different levels revealed absence of large myelinated fibers with a maximal axonal atrophy, which was progressive [1,30]. Up to 6 months of age, there was a significant accumulation of glucose, sorbitol and fructose in peripheral nerve tissue and DRG [33].

One-month treatment with gangliosides improved MNCV and axonal morphometry at 6 and 9 months of age db/db mice [30].

Other transgenic models used to study diabetic neuropathy mechanisms
Transgenic mice overexpressing aldose reductase (AR+/+)
To explore detailed mechanisms of how polyol pathway is involved in diabetic neuropathy and to develop specific inhibitors for human AR, transgenic mouse model overexpressing human AR is now available [34]. These animals exhibit neither glucose nor insulin level alterations.

Five-week duration of STZ-induced diabetes in AR+/+ mice showed a transient decrease of pain sensation threshold followed by a significant elevation after 12 weeks of diabetes [35]. From 4- to 12-week duration of STZ-induced diabetes in AR+/+ mice, MNCV and SNCV were reduced; sorbitol levels in the sciatic nerve and oxidative stress were increased [35,36]. Eight-week duration of STZ-induced diabetes in AR+/+ mice, protein kinase C activity in the sciatic nerve membrane was reduced, MNCV was severely decreased as well as a nerve fiber atrophy [34]. Overexpression of AR in Schwann cells led to a greater reduction in MNCV in the 8-week-old galactosemic mice, suggesting that metabolic imbalance in the Schwann cells is an important contributing factor to diabetic neuropathy [37].

STZ-AR deficiency (AR−/−) mice or ARI treatment in STZ-AR+/+ mice prevented neuropathy development [36].

Transgenic mice lacking peripheral axonal neurofilaments (NF-H-lacZ)
To explore the influence of neurofilament on diabetic neuropathy development, NF-H-lacZ mice rendered diabetic (STZ) were used [38]. Eight-week-old diabetic mice lacking axonal neurofilaments had a larger reduction in NCV, declined nerve action potential amplitudes and slight increased axonal atrophy [38]. Insulin injection increased NCV and axon caliber in these mice.

Transgenic mice expressing fluorescent sensory/motor neurons (thy-YFP)
All of the sensory/motor neurons in thy-YFP mice appeared bright yellowish-green when viewed by fluorescence stereomicroscopy [39], and when the hair was removed, cutaneous nerves were visible. In diabetic thy-YFP mice, loss of heat-induced pain perception occurred as early as 1-month duration of diabetes and followed at 3 months of diabetes by a significant loss of small cutaneous nerve fiber [39]. Impairment of sensory nerves preceded cutaneous nerve fiber loss.

Transgenic and knockout models in other pathways regulating insulin action
Mouse models with single genetic defects in insulin action can yield important information about the importance of insulin resistance in diabetic neuropathy occurrence. All these models have been very well reviewed [40].

Model comparison
The decision regarding which rodent model of diabetes to use for any particular experiment is often multifactorial. Ideally, experiments should be carried out in several different types of animal although, in practice, individual research groups tend to build up experience with one strain.

NOD mouse seems particularly analogous to human type 1 diabetes. However, the unpredictable age at the onset of diabetes that can occur from 12 to 30 weeks renders any comparison with other type 1 animal models even more difficult (Table 1). STZ animals are highly hyperglycemic leading to severely ill condition, exacerbating all pathological mechanisms.

In addition, daily administration of exogenous insulin renders NOD and long-term STZ models complex for results comparison.

GK and ZDF rats better suit experimental studies of neuropathy of prediabetes rather than of type 2 diabetes, as both models either do not develop obvious fasting hyperglycemia (GK) or develop it very late (ZDF). In addition, mutation of leptin (ob/ob) and leptin receptor (db/db) is a very rare cause of obesity and type 2 diabetes in humans. BB/Wor-rats and BBZDR/Wor-rats are outbred from the same BB background providing unique comparison models representative of the two main forms of human diabetes. In transgenic models, knockout gene cannot be timed to occur during specific periods of the animal’s life.

Model translation to humans
There are likely differences between the complications seen in animal models and humans and debate exists as to
whether any currently available animal model accurately reflects the diabetic complications seen in man. In general, one animal model represents only one aspect or subtype of diabetes in humans and care must always be taken when extrapolating results to the clinical setting. Animal studies have the advantage of allowing the use of specific drugs that cannot be administered to humans to go further in the determination of the molecular mechanisms.

Conclusions
Diabetic neuropathy is a frequent and heterogeneous complication of diabetes mellitus, which itself is a highly complex pathology. Diabetic neuropathy does develop in several diabetic rodent models but in a different manner, depending on the strain, the type of diabetes, the age occurrence of diabetes and the diabetes duration. However, significant advances in diabetic neuropathy knowledge have arisen in recent years from these models, leading to the development of potential therapeutic agents that should provide better outcome in diabetic patients.

Table 1. Comparison summary table

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<th>Type 2 diabetes</th>
<th>Tissue or molecule-specific transgenic/knockout models</th>
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<td><strong>Pros</strong></td>
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<td>– Spontaneous b-cell failure may mimic pathophysiology of disease in humans</td>
<td>– Likely to be as complex and heterogeneous as the human condition</td>
<td>– Study of specific components of the pathogenesis</td>
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<td>– Onset of diabetes can be determined by investigators (STZ)</td>
<td>– Several strains and models mimicking different aspects of the pathology</td>
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<td><strong>Cons</strong></td>
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<td></td>
<td>– Unpredictable onset of diabetes on spontaneous strains leading to variable age</td>
<td>– Mutation of leptin and leptin receptor is a very rare cause of obesity and type 2 diabetes in humans</td>
<td>– Gene inactivation is effective throughout development leading to possible compensatory mechanisms</td>
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<td>– Drug-induced tissue toxicity</td>
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<td>– Severe hyperglycemia not comparable to humans</td>
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<td>– Insulin administration for long-term studies in spontaneous and STZ models</td>
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<td><strong>Best use of model</strong></td>
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<td>– Direct effect of hyperglycemia on diabetic neuropathy</td>
<td>– Neuropathy in pre-diabetic state</td>
<td>– To better understand factors that exacerbate diabetic neuropathy</td>
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<td></td>
<td>– Severe diabetic neuropathy</td>
<td>– Progressive development of diabetic neuropathy</td>
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References
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