Diabetic Neuropathy in Spontaneously Diabetic Torii Rat

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Abstract: To aid in the study of diabetes and its complications, many diabetic animal models have been reported. Although most diabetic patients suffer type 2 diabetes, studies using type 2 diabetic animal models have been carried out less frequently. Spontaneously Diabetic Torii (SDT) rat, a non-obese type 2 diabetes model, shows neuropathies and severe ocular complications. Decreased nerve conduction velocity and thermal hypoalgesia were improved by insulin treatment, indicating that the peripheral neuropathies in SDT rats are caused by sustained hyperglycemia. Autonomic nerve dysfunctions such as decreased coefficients of variance of R-R intervals (CV_{R-R}) in electrocardiogram, functional gastrointestinal disorders, and voiding dysfunction are also observed in SDT rats. Therefore, SDT rat is a useful diabetic animal model for studies of diabetic neuropathies in type 2 diabetes and development of new drugs and therapies for diabetic neuropathies.

INTRODUCTION

Diabetes mellitus (DM) is one of the most common diseases, and more than half of all DM patients have diabetic complications such as diabetic retinopathy, diabetic nephropathy, or diabetic neuropathy. Among these complications, diabetic neuropathy is the most frequent and up to 50% of diabetics suffer some form of nerve damage [1]. Moreover, diabetic neuropathy causes foot ulceration and may lead to amputation, and may also lead to chronic pain with reduced quality of life. Most common among the diabetic neuropathies are diabetic peripheral neuropathy (DPN) and the diabetic autonomic neuropathy (DAN). Large clinical trials have indicated that strict control of blood glucose level in both type 1 and type 2 diabetes patients can delay the onset and progression of these diabetic neuropathies [2, 3].

To study DM and its complications, many diabetic animal models have been reportedly tested [4-8]. These animal models showing hyperglycemia were followed by complications in several tissues, including kidneys, nerves, and eyes. Spontaneously Diabetic Torii (SDT) rat is a new model for non-obese type 2 diabetes [9, 10]. Although most DM patients suffer type 2 diabetes, studies using type 2 diabetic animal models have been carried out less frequently [11, 12]. Therefore basic research using type 2 diabetic animal models is necessary for the development of new drugs and treatments for diabetic neuropathy. SDT rat shows marked hyperglycemia (Fig. **1A**) and subsequent severe ocular complications [9, 10, 13-15] and nephropathy [16]. On the other hand, although SDT rats show all three major diabetic complications, only a few reports on neuropathies have been published [17-20]. Therefore in this article,

characteristics of diabetic neuropathies in SDT rats are overviewed.

DIABETIC PERIPHERAL NEUROPATHY

Peripheral nerve fibers are classified as large or small fibers. Motor nerve conduction velocity (MNCV) and sensory nerve conduction velocity (SNCV) defects in diabetic animals are markers of large myelinated fiber dysfunction [21, 22]. Wada *et al.* first reported the MNCV in SDT rats [19, 20]. In their report, SDT rats showed decreased MNCV around 30 weeks of age. MNCV was decreased to 82% that of normal rats at 40 weeks of age and to 76% at 48 weeks of age (Fig. **1B**). When the blood glucose levels were strictly controlled by insulin for 24 weeks, disorder in tail MNCV was significantly prevented (Fig. **2A**, **B**). Therefore, it is clear that the decreased MNCV was caused by sustained hyperglycemia [18].

Small fibers (*e.g.* Aδ and C-fiber) mediate sensation of temperature and pain as well as autonomic functions. Both hyper- and hypoalgesia are found in diabetic state. Duration of hyperglycemia, the severity of diabetic state, sex or species may affect progression to diabetic hyper- or hypoalgesia. While thermal hypoalgesia is reported in diabetic animals using tail-flick test or hot plate test [23-25], others have found hyperalgesia [26-28]. At 32 weeks of age, marked thermal hypoalgesia was observed in SDT rats by tail-flick test [17].

DIABETIC AUTONOMIC NEUROPATHY

Autonomic nerve functions are also impaired in diabetes. Coefficient of variance of R-R intervals (CV_{R-R}) in electrocardiogram, a marker of autonomic nerve function, was significantly lower in diabetic SDT rats (44 weeks of age) compared to age-matched normal Sprague-Dawley (SD) rats (SDT rats have the SD rats as their background), indicating disorder of autonomic nerve function [17]. Twenty-four week administration of insulin prevented worsening of CV_{R-R} in SDT rats (Fig. **2C**) [18]. The

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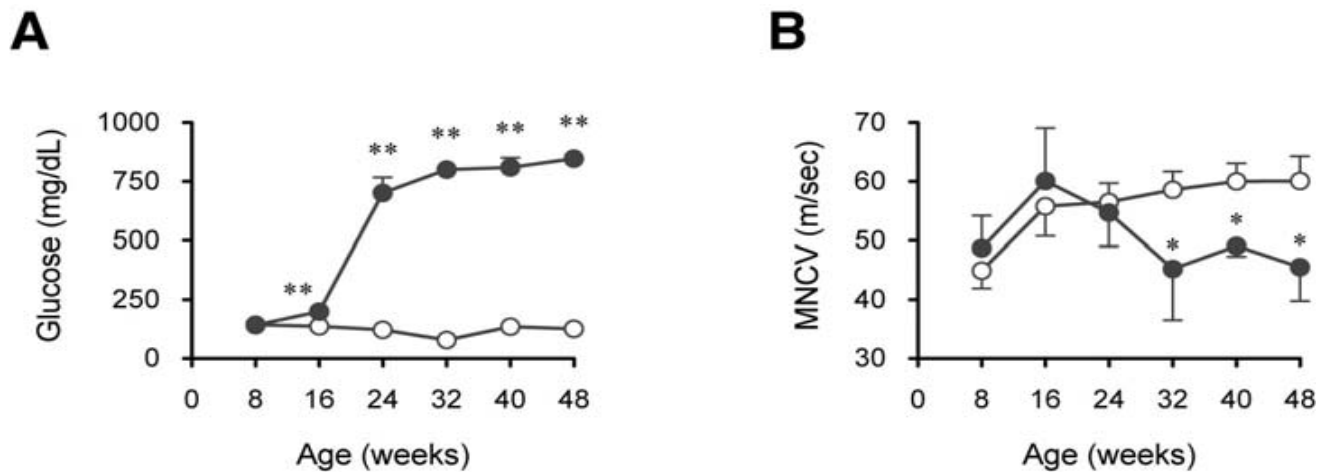
Fig. (1). Serial changes of (**A**) blood glucose level and (**B**) tibial motor nerve conduction velocity (MNCV) in SD rats (open circles) and SDT rats (closed circles). Figure B was kindly provided by Dr. Wada (Hirosaki University School of Medicine). Data represent means ± S.E.M. (n=4.6). *p<0.05, **p<0.01 (*vs* age-matched SD rat, unpaired *t*-test for (**A**), Mann-Whitney U-test for (**B**)).
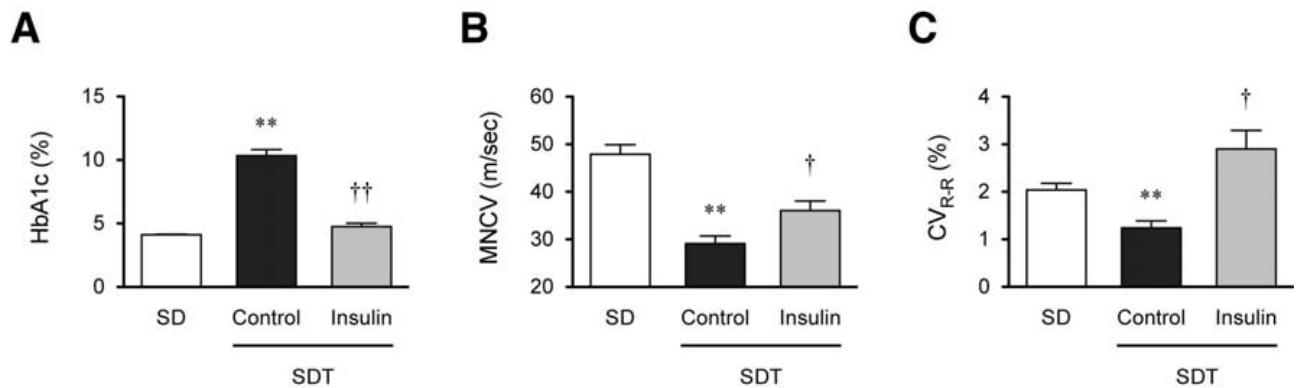

Fig. (2). Effect of blood glucose control by insulin on (**A**) HbA1c, (**B**) tail motor nerve conduction velocity (MNCV) and (C) coefficients of variance of R-R intervals (CV_{R-R}) in electrocardiogram. Twenty four-weeks old SDT rats were treated with insulin pellets for 20 weeks. Data represent means ± S.E.M. (n=3-6). **p<0.01 (*vs* age-matched SD rat, unpaired *t*-test), †p<0.05, ††p<0.01 (*vs* control SDT rat, unpaired *t*-test).

impaired autonomic nerve is deeply associated with diabetic gastroenteropathy. Functional gastrointestinal disorders such as rapid gastrointestinal transit and diarrhea are observed in SDT rats [29]. As several reports on DAN have shown [30, 31], insulin therapy is effective on these diabetic gastrointestinal abnormalities in SDT rats [29]. Voiding dysfunction is also observed as a result of DAN. Bladder dysfunctions such as diabetic cystopathy are common symptoms among diabetic patients [32] and diabetic animal models [33] with DAN. Reduced production of nerve growth factor (NGF) in the bladder and dorsal root ganglia (DRG) was associated with voiding dysfunction attributable to defects in Aδ and C-fiber bladder afferents in streptozotocin (STZ)-induced diabetic rats [34]. In SDT rats, increase in voided volume and inter-micturition interval related to the afferent limb neuropathy and the increase in voiding pressure related to urethral dysfunction were observed as diabetic bladder dysfunction [35].

HISTOPATHOLOGY

The histopathological characteristics of sural nerve in SDT rats are described by Wada *et al.* [19, 20]. Although

neurologic deficits were not observed in sural nerve, incidence of degenerating nerve was increased in SDT rats. Morphologically, SDT rats revealed significant atrophy in myelinated nerve at 48 weeks of age: cross-sectional area was 88% that of normal rats. At this age, the number of small fibers was relatively increased in SDT rats. The number of endoneural blood vessels in SDT rats was comparable with that in normal rats; however, occluded vessels were found in SDT rats (Fig. **3**). Considering that the main pathological findings in human diabetic nerves consist of progressive fiber loss and endoneural microangiopathy [5], SDT rat is a useful animal model of type 2 diabetes.

TREATMENT OF DIABETIC NEUROPATHY

Multiple mechanisms have been implicated in diabetic complications including diabetic neuropathies: increased polyol pathway, hexosamine pathway, and advanced glycation endproducts (AGEs) are well-known factors. Activation of protein kinase C beta (PKCβ), through the *de novo* synthesis of diacylglycerol (DAG) is also observed under diabetic conditions. Although hyperglycemia is a
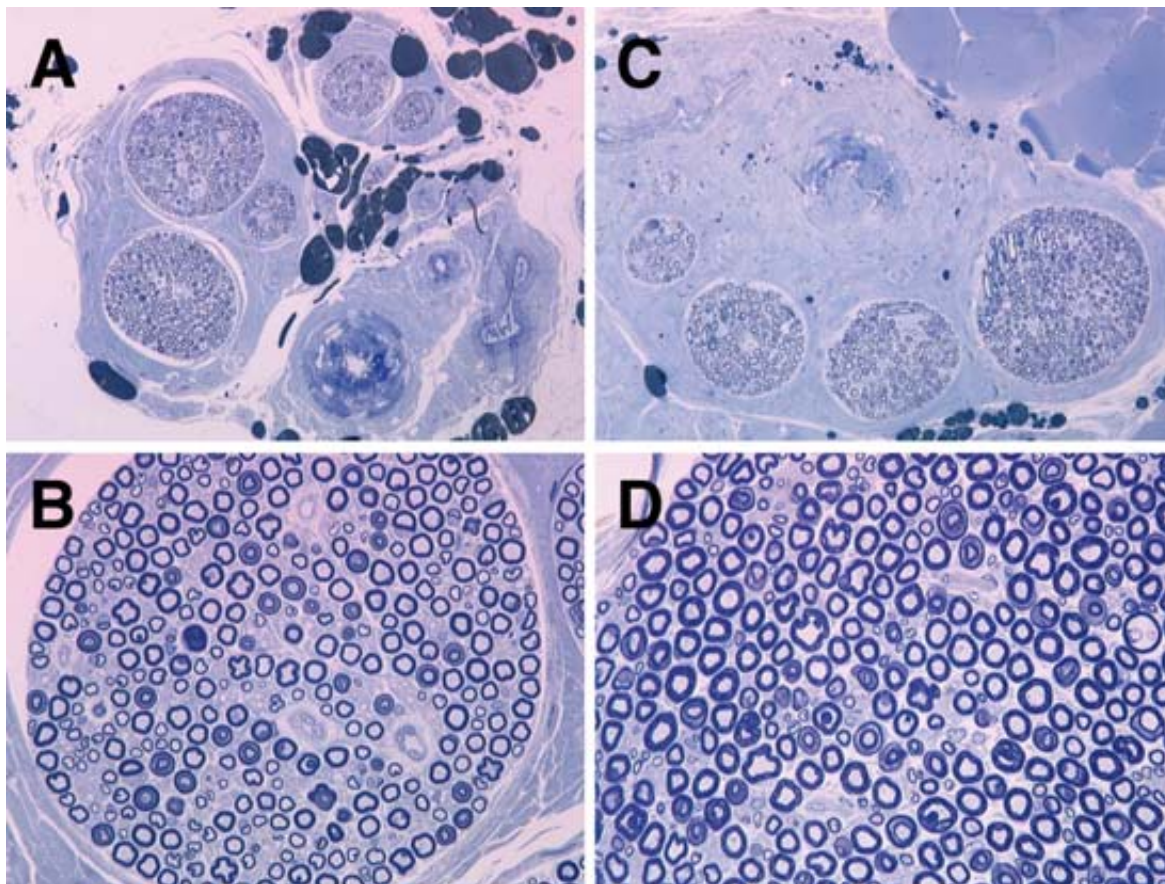
Fig. (3). Structural changes of the peripheral nerves. (**A**) Low and (**B**) high magnification of sural nerve from 12 months old SD rat. (**C**) Low and (**D**) high magnification of sural nerve from 12 months old SDT rat. Toluidine blue stain. Microphotographs were kindly provided by Dr. Wada.

major determinant of diabetic complications, current oral anti-hyperglycemic drugs have not shown sufficient efficacy to prevent the development of diabetic microvascular complications. Therefore, novel drugs for diabetic complications independent from hypoglycemic effect are desperately needed. Aldose reductase inhibitor (ARI) and PKCβ inhibitor were proven to prevent diabetic retinopathy without controlling blood glucose levels in SDT rats [17, 36]. Long-term treatment of a PKCβ selective inhibitor, JTT-

010, prevented diabetic neuropathy in SDT rats [17]. Twelve-week administration of JTT-010 prevented delay of MNCV, and decreased CV_{R-R} and thermal hypoalgesia in SDT rats without affecting blood glucose levels. However, JTT-010 did not prevent hyperglycemia-induced retinal abnormalities in SDT rats (*e.g.* abnormal retinal vascular formation, protruded optic disc). Therefore, it seems that the different factor(s) contribute to progression of diabetic neuropathy and diabetic retinopathy in SDT rats.
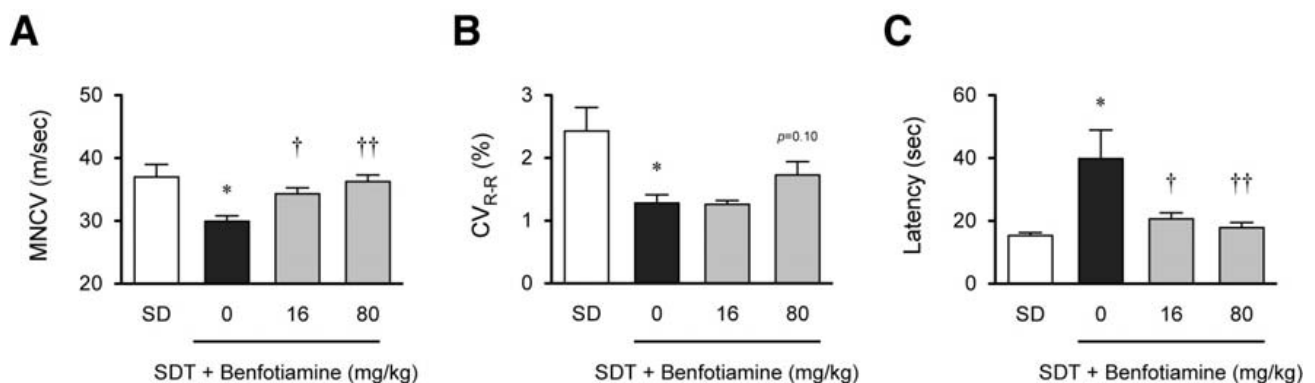

Fig. (4). Effect of benfotiamine on (**A**) tail MNCV, (**B**) CV_{R-R}, and (**C**) thermal hypoalgesia (tail-flick test). Twenty-weeks old SDT rats were treated with benfotiamine by food admixture for 12 weeks. Data represent means ± S.E.M. (n=7-8). *p<0.05 (*vs* SD rat, unpaired *t*-test), †p<0.05, ††p<0.01 (*vs* untreated SDT rat, Dunnett's test).

Benfotiamine reduces all four major pathways (polyol pathway, hexosamine pathway, AGE pathway, and DAG-PKC pathway) by activating transketolase, a thiamine-dependent pentose phosphate pathway enzyme. This liposoluble prodrug of thiamine exhibits effects on peripheral nerve function as well as on diabetic retinopathy [37, 38]. Benfotiamine clearly showed blood glucose-independent effect on DPN/DAN in SDT rats, as well as blood glucose control by insulin, indicating that the SDT rat is a helpful model for research and development of new pharmaceuticals. Twelve-week treatment of benfotiamine (20-32 weeks of age) improved MNCV with dose dependency. Administration of benfotiamine also tended to ameliorate CV_{R-R} and prevented elevation of tail-flick threshold significantly (Fig. **4**). Biochemical parameters and body weight of SDT rats were not affected by benfotiamine.

CONCLUSION

In conclusion, neuropathies in SDT rats are caused by sustained hyperglycemia and therefore SDT rat is useful diabetic animal model for studies in diabetic neuropathies in type 2 diabetes and for the development of new drugs and therapies for diabetic neuropathies.

ACKNOWLEDGEMENTS

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