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Tolrestat Improves Nerve Regeneration After Crush Injury in Streptozocin-Induced Diabetic Rats

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To delineate the ability of diabetic nerves to regenerate and to determine the effect of aldose reductase (AR) inhibitors (ARIs) on nerve regeneration in diabetic neuropathy, we evaluated nerve regeneration electrophysiologically and morphologically after sciatic nerve crush injury in three groups of male Sprague-Dawley rats: untreated diabetic (streptozocin [STZ]-induced, n = 16), tolrestat-treated diabetic (n = 16), and age-matched controls (n = 16). Compound muscle action potentials (CMAPs) appeared 4 weeks after crush injury in the control group and 5 weeks after injury in both diabetic groups. Motor nerve conduction velocity (MNCV) in the crushed nerves was decreased in both diabetic groups compared with the control group throughout the experiment. However, this decrease was significantly prevented at 24 weeks with tolrestat treatment. Morphologically, the density of myelinated nerve fibers (MNFs) and the number of MNFs per fascicle were significantly decreased in untreated diabetic rats, but tolrestat significantly prevented the former decrease at 5 weeks and the latter at 24 weeks. The mean diameter of large MNFs (>4 μm) was smaller in the untreated diabetic group than in the control group, but this decrease also was significantly prevented with tolrestat treatment. These results suggest that nerve regeneration is impaired in diabetic neuropathy and that tolrestat can prevent this impairment.

ALTHOUGH DIABETIC neuropathy is one of the most common forms of peripheral neuropathy, its pathogenesis remains unclear. Recently, several hypotheses have been proposed to explain the pathogenesis of diabetic neuropathy, and therapeutic agents have been developed on the basis of these theories. Based on the hypothesis that nerve dysfunction in diabetic neuropathy is attributable to increased polyol accumulation,[1] decreased *myo*-inositol content,[2] and an associated decrease in Na^+/K^+-adenosine triphosphatase activity in peripheral nerves,[3] aldose reductase (AR) inhibitors (ARIs) have been used to treat diabetic neuropathy and are reported to prevent and/or reverse both the decreases in nerve conduction velocity and the abnormalities in polyol metabolism in diabetic animals and patients.[4-6] On the other hand, based on the hypothesis that microvascular disease is the cause of diabetic neuropathy,[7-11] vasotropic compounds, including prostaglandin E_1 and its analogs, have been used to treat it.[12-14]

The goal in treating diabetic neuropathy is not only to prevent the progression of neuropathic symptoms and nerve dysfunction[15,16] but also to promote the regeneration of degenerated nerve fibers. Since the recovery of decreased nerve function depends on both the regeneration of degenerated nerve fibers and the reestablishment of their functional connections with muscles and skin, it is important to examine how the diabetic condition influences nerve regeneration and whether potential therapeutic agents can promote the satisfactory regeneration of functional nerve fibers.

Recently, the ARIs, sorbinil and tolrestat, have been reported not only to improve clinical symptoms and neurophysiologic function but also to increase nerve regeneration in human diabetic neuropathy.[5,17] Although these reports provide strong evidence that ARIs may be potent promoters of nerve regeneration in diabetic neuropathy, an animal model is necessary to precisely and objectively evaluate the ability of potential therapeutic agents to promote nerve regeneration. However, although some animal models, including BB Wistar rats, have been reported to show considerable nerve fiber loss,[18] the magnitude of the fiber

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loss does not mimic human diabetic neuropathy.[19] Furthermore, nerve fiber loss takes longer to occur in these animals, and thus more time is required to evaluate the specific effects of test compounds on nerve regeneration. In this sense, the nerve-crush model may be useful in studying nerve regeneration, since nerve regeneration after a crush injury occurs in a predictable manner.[20]

The aims of this study were to use electrophysiologic and histologic techniques to confirm that nerve regeneration is indeed decreased in streptozocin (STZ)-induced diabetic rats, and to ascertain whether tolrestat, a potent ARI,[21] improves this defect in nerve regeneration.[22-24]

MATERIALS AND METHODS

Experimental Animals and Research Design

Thirty-two adult male Sprague-Dawley rats aged 9 weeks were rendered diabetic with a single intravenous injection of STZ 50 mg/kg dissolved in 0.1 mol/L citrate buffer (pH 4.2) into the tail vein. Another 16 rats were administered 1 mL citrate buffer via the tail vein and served as age-matched controls.

We performed nerve conduction and morphometric studies on three groups of experimental animals, untreated diabetic, tolrestat-treated diabetic, and control rats, using separate experimental protocols. One week after STZ injection, the animals were divided randomly into these three groups. We used 12 diabetic (six untreated and six tolrestat-treated) and six control rats for the nerve conduction studies, and 20 diabetic (10 untreated and 10 tolrestat-treated) and 10 control rats for the morphometric studies. Control and untreated diabetic rats were fed standard rat chow. Tolrestat-treated diabetic rats were fed standard rat chow mixed with tolrestat (25 $mg \cdot kg^{-1} \cdot d^{-1}$). We adjusted the concentration of tolrestat once per week by monitoring food consumption throughout the experiment, to administer the experimental animals a constant dose of tolrestat. The tolrestat treatment was started after the crush injury in the right sciatic nerve, which was performed at 10 weeks of age in all rats.

Motor nerve conduction was measured in five to six rats from each group 1, 2, 3, 4, 5, 10, 14, 19, and 24 weeks after the sciatic nerve crush injury. This measurement was repeated in the same rat throughout the experimental period. A morphometric examination was performed in another four to five rats from each group 5 and 24 weeks after the crush injury. During the experiment, two tolrestat-treated diabetic rats and two untreated diabetic rats died of ketoacidosis and one control rat died of an overdose of anesthetic during a nerve conduction study. Blood glucose concentrations were determined using a reflectance dextrometer (TOYOBO, Tokyo, Japan).

Nerve Crush

The experimental animals were anesthetized with 35 mg/kg intraperitoneal pentobarbital sodium. The right sciatic nerve was exposed in the upper thigh, and a crush approximately 1.0 mm wide was made with forceps for 30 seconds 5 mm distal to the sciatic notch. The crush point was marked with an epineural suture. To minimize variability in the extent of the nerve injury induced by the crush, the same person performed the nerve crush for the same period and with the same forceps. This method has been used by other researchers.[25,26]

Nerve Conduction Study

Nerve conduction measurements of the regenerating nerve fibers were performed with a Medelec MS-92B (England) on the right hindleg, which was held in full extension with straps, under anesthesia with intraperitoneal pentobarbital sodium (35 mg/kg). The sciatic nerve was stimulated at the sciatic notch (proximal to the crush site) and the tibial nerve was stimulated posterior to the ankle with a steel needle electrode using supramaximal stimulation through the skin. The anode needle electrode was inserted into the gluteal region. Compound muscle action potentials (CMAPs) were recorded from the plantar muscle of the right hindleg with a unipolar needle electrode. Motor nerve conduction velocity (MNCV) and the peak-to-peak amplitude of the CMAP were measured. MNCV studies also were performed on the uncrushed side using the same procedure. The body temperature was monitored with a rectal probe, and the core temperature was maintained at 37.0° ± 1.0°C with a heating pad and lamp (Nihon Kohden, Tokyo, Japan).

Morphometry

The crushed sciatic nerves were reexposed and fixed in situ in 2.5% glutaraldehyde/2% paraformaldehyde in 0.025 mol/L cacodylate buffer (pH 7.38) for 60 minutes. The sciatic nerves were then removed and fixed with the same fixative at 4°C for 3 hours. The tissue blocks were additionally fixed in 1% osmium tetroxide and embedded in epoxy resin. Transverse semithin sections (1 μm) 5 mm distal to the crush site were stained with toluidine blue. Morphometric analyses were performed with a computer-assisted digitizer (KONTRON IBAS-1, Germany). We measured the myelinated nerve fibers (MNFs) on three randomly selected square areas (total, 0.104 mm^2) within a fascicle, resulting in the measurement of approximately one fourth of all fibers per fascicle, on average. The following parameters were obtained: (1) MNF density (MFD), (2) number of MNFs per fascicle (no. MNF), (3) mean diameter of MNFs, (4) mean diameter of large MNFs (> 4 μm in diameter), and (5) ratio of MNFs larger than 4 μm in diameter to total MNFs (% large fiber). In addition, size-frequency histograms of MNFs were made. MNFs assessed 5 and 24 weeks after crush injury were regenerating MNFs, because all MNFs distal to the crush site underwent Wallerian degeneration and immediately regenerated.

Statistical Analysis

All results are expressed as the mean ± SD. Comparisons between groups were made by one-way or two-way ANOVA, followed by Scheffe's test. P less than .05 was considered statistically significant.

RESULTS

Clinical Observation

Baseline body weight and plasma glucose concentration were not different between control and experimental groups in either the nerve conduction or morphometric studies. In both studies, plasma glucose concentrations were significantly higher and body weights were significantly lower in both diabetic groups than in the control group (Tables 1 and 2). Tolrestat did not affect body weight or plasma glucose concentration, nor did it have any other apparent adverse effect.

Nerve Conduction Study

CMAPs of crushed nerves first appeared 4 weeks after the injury in all control rats and at 5 weeks in all diabetic rats in both groups. During the experiment, there was no significant difference in CMAP amplitude between any of

Table 1. Clinical Characteristics of Experimental Animals for Nerve Conduction Study

Characteristic	−1 Week	5 Weeks	14 Weeks	24 Weeks
Body weight (g)				
Control (n = 6)	308 ± 8	401 ± 25	487 ± 16	520 ± 23
DM (n = 6)	297 ± 8	288 ± 22	296 ± 24	300 ± 21
DM + ARI (n = 5)	308 ± 11	292 ± 24	308 ± 41	310 ± 29
Plasma glucose (mg/dL)				
Control (n = 6)	112 ± 10	112 ± 7	115 ± 10	116 ± 11
DM (n = 6)	113 ± 9	440 ± 56	467 ± 44	453 ± 59
DM + ARI (n = 5)	112 ± 10	459 ± 53	494 ± 81	461 ± 37

NOTE. Results are the mean ± SD. Two-way ANOVA: $P < .0001$ for experimental group, time, and interaction between the 2 factors. Scheffe's test: control *v* DM, $P < .0001$; control *v* DM + ARI, $P < .0001$; DM *v* DM + ARI, NS.

Abbreviations: DM, untreated diabetic rats; DM + ARI, tolrestat-treated diabetic rats.

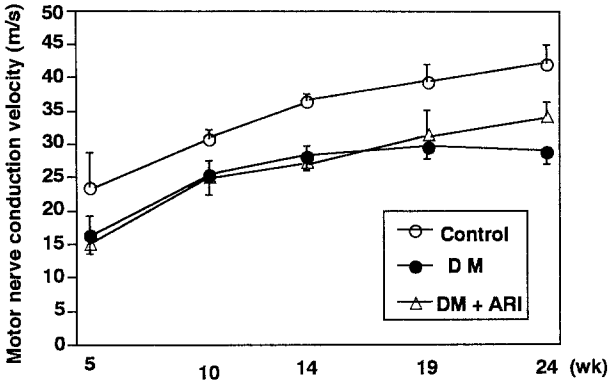

Fig 1. MNCV of crushed sciatic nerve in control, untreated diabetic (DM), and tolrestat-treated diabetic (DM + ARI) rats. The effect of tolrestat on reducing MNCV in DM rats was maximal at 24 weeks. Vertical lines represent standard deviations. Two-way ANOVAs for experimental group and time are $P < .0001$; Scheffe's test: control *v* DM, $P < .0001$; control *v* DM + ARI, $P < .001$; DM *v* DM + ARI, $P < .05$.

the groups, except for control versus tolrestat-treated diabetic rats (Table 3). The MNCV of crushed nerves was slower in both diabetic groups than in control throughout the experiment. However, tolrestat provided a small but significant improvement in MNCV at 24 weeks (Table 3 and Fig 1).

The MNCV of uncrushed nerves in untreated diabetic rats was significantly slower than in control rats throughout the experimental period. Tolrestat completely prevented this reduction in MNCV (Table 3 and Fig 2).

Morphometric Study

The mean fascicle area was not different among the three groups at 5 and 24 weeks, indicating that the extent of endoneurial edema in the regenerating nerve was not different among the groups. MFD and no. MNF were decreased significantly in untreated diabetic rats compared with control rats at 5 and 24 weeks. Tolrestat completely prevented the reduction in MFD at 5 weeks and partially but significantly prevented the reduction in no. MNF at 24 weeks. Although tolrestat also had a small but positive effect on the reduction in MFD at 24 weeks and the reduction in no. MNF at 5 weeks, these were not statistically significant. Mean nerve diameter was not different among the three groups at 5 and 24 weeks. However, the percentage of large fiber was significantly lower in untreated diabetic rats than in control rats at 5 and 24 weeks. Tolrestat ameliorated this decrease, but it was not statistically significant. On the other hand, the mean diameter of regenerating MNFs larger than 4 μm was smaller in untreated diabetic rats than in control rats, and this reduction was significantly prevented by tolrestat (Table 4). Size-frequency histograms of MNFs after crush injury showed that at 24 weeks the densities of small and large MNFs were significantly lower in untreated diabetic rats than in control rats. In tolrestat-treated diabetic rats, the densities of small and large MNFs were greater than in untreated diabetic rats at 24 weeks (Fig 3).

Table 2. Clinical Characteristics of Experimental Animals for Morphometric Study

Characteristic	−1 Week	5 Weeks	24 Weeks
Body weight (g)			
Control	311 ± 10 (9)	400 ± 30 (4)	539 ± 19 (5)
DM	306 ± 9 (8)	302 ± 6 (4)	301 ± 11 (4)
DM + ARI	310 ± 9 (9)	298 ± 5 (5)	304 ± 14 (4)
Plasma glucose (mg/dL)			
Control	113 ± 9 (9)	112 ± 10 (4)	107 ± 16 (5)
DM	110 ± 9 (8)	510 ± 84 (4)	500 ± 24 (4)
DM + ARI	110 ± 12 (9)	473 ± 59 (5)	446 ± 47 (4)

NOTE. Parentheses indicate number of animals per group. One-way ANOVA: $P < .0001$ at 5 and 24 weeks. Scheffe's test at 5 weeks: control *v* DM, $P < .0001$; control *v* DM + ARI, $P < .0001$; DM *v* DM + ARI, NS. Scheffe's test at 24 weeks: control *v* DM, $P < .0001$; control *v* DM + ARI, $P < .0001$; DM *v* DM + ARI, NS.

DISCUSSION

In the present study, nerve conduction measurements showed that the reappearance of CMAPs after a crush injury was delayed by 1 week in untreated diabetic rats compared with control rats. CMAPs after a nerve crush injury do not reappear until regenerating MNFs have reached their target organ. This delay may be attributed to a reduced rate of axonal growth or impaired reestablishment of the neuromuscular junction in diabetes. Previous reports have documented that the rate of axonal growth after a crush injury is decreased in STZ-diabetic rats.[22,23]

The MNCV of the crushed nerve was persistently and significantly decreased in untreated diabetic rats compared with control rats. The finding that the percentage of fibers larger than 4 μm was lower in untreated diabetic rats than in control rats is compatible with the results of the nerve conduction study. These findings suggest that the maturation process of regenerating MNFs is impaired in diabetes.

In the morphologic studies, a decreased number of regenerating MNFs after a crush injury was shown in diabetic rats, as previously reported by Triban et al[24] on the

Table 3. Electrophysiologic Findings in Crushed and Uncrushed Sciatic Nerve of Control, Untreated Diabetic, and Tolrestat-Treated Diabetic Rats 5, 10, 14, 19, and 24 Weeks After Crush Injury

Parameter	5 Weeks	10 Weeks	14 Weeks	19 Weeks	24 Weeks
MNCV of crushed sciatic nerve (m/s)*					
Control (n = 6)	25.8 ± 4.0	31.1 ± 1.2	36.7 ± 1.0	39.6 ± 2.4	42.0 ± 3.2
DM (n = 6)	17.5 ± 1.9	25.6 ± 3.1	28.2 ± 2.2	29.8 ± 2.1	29.0 ± 2.0
DM + ARI (n = 5)	16.3 ± 3.7	25.4 ± 1.9	27.6 ± 2.1	31.8 ± 3.3	34.4 ± 2.1
MNCV of uncrushed sciatic nerve (m/s)†					
Control (n = 6)	55.2 ± 1.0	55.4 ± 2.1	55.7 ± 3.1	56.3 ± 2.5	58.9 ± 1.6
DM (n = 6)	48.0 ± 2.0	49.8 ± 2.4	50.7 ± 2.8	51.6 ± 3.3	51.0 ± 1.0
DM + ARI (n = 5)	52.1 ± 4.1	54.9 ± 1.6	57.1 ± 3.2	59.4 ± 2.6	58.2 ± 1.7
CMAP amplitude of the crushed side (mV)‡					
Control (n = 6)	1.5 ± 0.8	10.4 ± 3.2	17.7 ± 4.9	17.1 ± 3.2	18.9 ± 4.9
DM (n = 6)	1.2 ± 0.2	8.6 ± 2.1	12.3 ± 4.8	16.7 ± 4.3	16.8 ± 2.7
DM + ARI (n = 5)	1.0 ± 0.2	6.8 ± 2.1	14.0 ± 1.5	14.5 ± 3.1	16.1 ± 2.7

*Two-way ANOVA: $P < .0001$ for experimental group and time. Scheffe's test: control *v* DM, $P < .0001$; control *v* DM + ARI, $P < .001$; DM *v* DM + ARI, $P < .01$.

†Two-way ANOVA: $P < .0001$ for experimental group and time. Scheffe's test: control *v* DM, $P < .0001$; control *v* DM + ARI, NS; DM *v* DM + ARI, $P < .0001$.

‡Two-way ANOVA: $P < .0001$ for experimental group and time. Scheffe's test: control *v* DM, NS; control *v* DM + ARI, $P < .01$; DM *v* DM + ARI, NS.

basis of immunohistochemistry. Tolrestat ameliorated the MNCV at 24 weeks after the crush injury in diabetic rats, although it did not affect the time at which the CMAP first appeared after the crush injury or the MNCV prior to 19 weeks. Ekstrom and Tomlinson[22] have previously demonstrated that an ARI (ponalrestat) did not restore the reduced regeneration rate in STZ-diabetic rats. This finding is compatible with the results of the present study. On the other hand, tolrestat prevented the reduced number and size of regenerating MNFs in diabetic rats after the crush injury throughout the experiment. Kamijo and Preston[27] also have shown that the reduced MNF regeneration was restored following ARI treatment (WAY 121509) in BB/W rats. The reason for the discrepancy between electrophysiologic and morphologic results prior to 24 weeks may be that the nerve conduction study evaluated motor nerve function, whereas morphometry was conducted on sciatic nerves, which have both motor and sensory function. It is also possible that some of the regenerating MNFs did not convey stimuli to muscles. Moreover, MNCV may evaluate the function of only the largest nerve fibers, which does not reflect the morphology of all the nerve fibers.

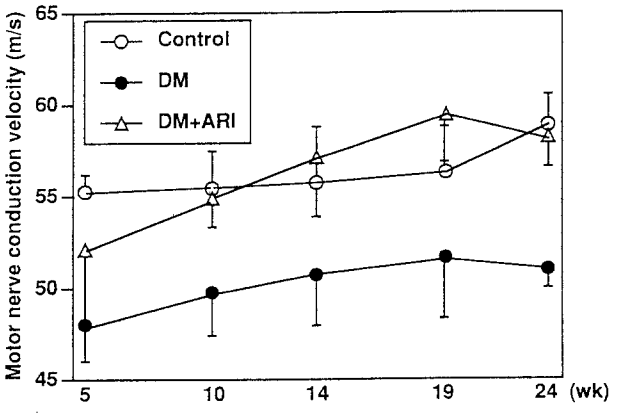

Fig 2. MNCV of uncrushed sciatic nerve in control, untreated diabetic (DM), and tolrestat-treated diabetic (DM ± ARI) rats. MNCV of uncrushed sciatic nerve was significantly reduced in DM *v* control rats throughout the experimental period. Treatment with tolrestat completely prevented this reduction throughout the experimental period. Vertical lines represent standard deviations. Two-way ANOVA for experimental group and time are $P < .0001$. Scheffe's test: control *v* DM, $P < .0001$; control *v* DM + ARI, nonsignificant; DM *v* DM + ARI, $P < .0001$.

On the other hand, it is interesting that tolrestat improves nerve conduction velocity in uncrushed nerves after just 5 weeks, whereas it has no positive effect on crushed nerves until 19 weeks later. Wong et al[28] have reported interesting results about a change in AR after a crush injury. AR activity was reduced significantly in the distal segment of crushed nerves during nerve degeneration. Although it increased during nerve regeneration, it had not returned to the previous level at 35 days after the crush injury. Therefore, AR is sufficiently reduced in both the control and diabetic crushed nerves that no further advantage is obtained from inhibiting AR during the first 19 weeks. However, after 19 weeks, regeneration is impaired in the diabetic nerves and AR inhibition has some value, perhaps because AR has exceeded the low levels at 35 days after the crush injury.

In the present study, we found that tolrestat ameliorated the nerve conduction deficit of uncrushed motor nerves. By contrast, Calcutt et al[29] have reported that tolrestat did not prevent a conduction deficit in diabetic rats. This discrepancy in the results of the two studies may be due to differences in the experimental animals and methods, including the age of the animals, the daily dose of tolrestat (25 mg/kg in our study *v* 50 mg/kg in Calcutt et al), and the method of feeding tolrestat. Moreover, Calcutt et al exposed the sciatic nerve to monitor nerve temperature in the nerve conduction study.

Furthermore, Calcutt et al[30] have reported that tolrestat disturbs nerve regeneration after crush injury in both control and galactose-fed hyperglycemic rats. Unfortunately, we did not study its effect on control rats. In our study, it had no effect on the decreased rate of nerve sprouting in diabetic rats. A number of factors may explain

Table 4. Morphometric Findings in Crushed Sciatic Nerve of Control, Untreated Diabetic, and Tolrestat-Treated Diabetic Rats 5 and 24 Weeks After Crush Injury

Parameter	MFD (per mm²)	No. MNF	Mean Diameter (μm)	Mean Diameter >4 μm (%)	Large Fibers (%)	Fascicle Area (mm²)
5 weeks						
Control (n = 4)	16,401 ± 1,774*	7,440 ± 477†	2.76 ± 0.15	4.88 ± 0.06*	14.3 ± 1.5†	0.46 ± 0.06
DM (n = 4)	12,945 ± 242	5,583 ± 1,271	2.69 ± 0.22	4.55 ± 0.18	8.7 ± 4.2	0.43 ± 0.09
DM + ARI (n = 5)	16,124 ± 312*	6,878 ± 487	2.68 ± 0.21	4.88 ± 0.04*	12.7 ± 3.1	0.43 ± 0.02
ANOVA	$P < .001$	$P < .05$	NS	$P < .001$	$P < .05$	NS
24 weeks						
Control (n = 5)	18,339 ± 1,472*	8,441 ± 393*	3.81 ± 0.17	5.61 ± 0.1*	41.4 ± 2.9†	0.46 ± 0.03
DM (n = 4)	13,090 ± 1,760	5,888 ± 386	3.60 ± 0.18	5.08 ± 0.14	32.6 ± 6.4	0.45 ± 0.07
DM + ARI (n = 4)	15,608 ± 1,137	7,104 ± 530‡	3.68 ± 0.16	5.43 ± 0.06*	37.7 ± 4.1	0.43 ± 0.07
ANOVA	$P < .01$	$P < .0001$	NS	$P < .05$	$P < .05$	NS

*$P < .01$, †$P < .05$ *v* DM and ‡$P < .01$ *v* control by Scheffe's test after 1-way ANOVA.

the differences in the effect of tolrestat between the two studies. The daily dose of tolrestat was two times higher in the study by Calcutt et al. They also used galactose-fed hyperglycemic rats, whereas we used STZ-induced hyperglycemic rats. In addition, there were several distinct differences in nerve biochemical (Na^+-K^+ ATPase activity) and morphological (extent of endoneurial edema) conditions between the two models. Moreover, Calcutt et al estimated the regeneration of sensory nerves using a pinch-reflex test, and we evaluated the regeneration of motor nerves using nerve conduction. Therefore, each study evaluated different types of nerve fibers.

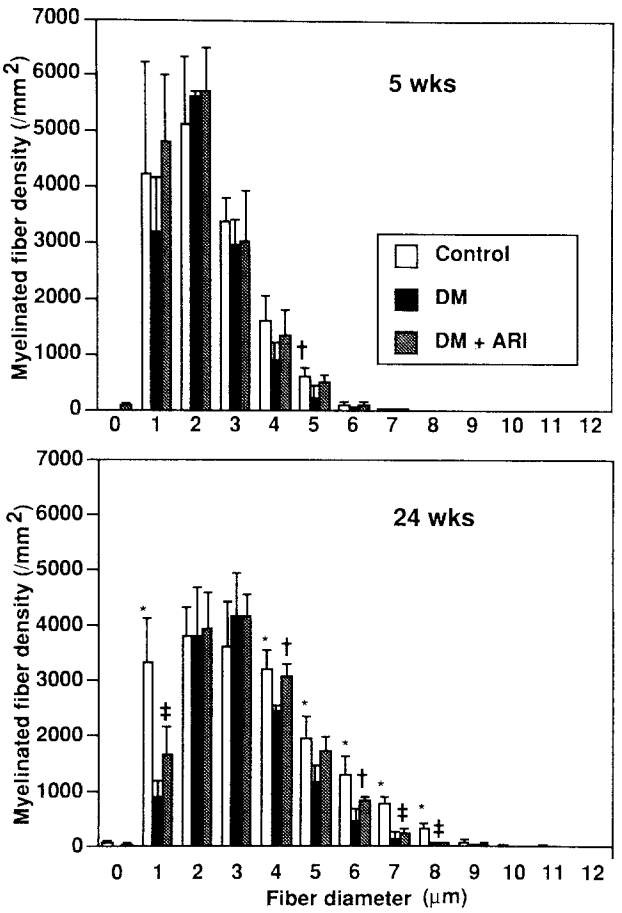

Fig 3. Size-frequency histograms of MNFs of crushed sciatic nerve in control, untreated diabetic (DM), and tolrestat-treated diabetic (DM + ARI) rats. MFDs of both small and large MNFS were decreased in DM *v* control rats at 24 weeks. Tolrestat significantly reversed this decrease at 24 weeks. Vertical lines represent standard deviations. One-way ANOVAs at both 5 and 24 weeks are $P < .0001$. Scheffe's test: *$P < .01$ and §$P < .05$ *v* DM; †$P < .01$ *v* control.

The potential mechanism by which tolrestat improves the number and size of regenerating nerve fibers is as follows. Axon caliber is determined by a number of cytoskeletal proteins such as neurofilament,[31] which is regulated by slow axonal transport.[32] This component of axonal transport has been reported to be decreased in STZ-diabetic rats and to be restored with ARI.[33] The synthesis of cytoskeletal proteins in the retina and the uptake of precursor amino acids in dorsal root ganglion cells also have been reported to be decreased in STZ-diabetic rats.[34,35] Furthermore, the induction of ornithine decarboxylase after a crush injury, which is closely associated with protein synthesis, has been reported to be decreased in the dorsal root ganglion cells of STZ-diabetic rats, and this decrease was prevented by the ARI ponalrestat.[36] In the present study, we found that tolrestat had no effect on the elongation rate of regenerating nerve fibers. Since the rate of nerve regeneration has been proposed to be correlated with slow component a, by which neurofilaments are carried,[37] tolrestat may increase the volume of slow axonal transport without affecting its velocity.

The effect of tolrestat on the number of regenerating MNFs might be mediated by neurotrophic factors, including nerve growth factor, insulin-like growth factors (IGFs), and ciliary neurotrophic factor (CNTF), which play important roles in the development and regeneration of the peripheral nervous system.[38-40] Nerve growth factor and CNTF and serum IGF-1 concentrations have been reported to be reduced in STZ-diabetic animals.[41-43] Recently, it also has been reported that IGF-1 and -2 gene expression is reduced in STZ-diabetic rats.[44] On the other hand, Schwann cells play an important role in nerve regeneration because they synthesize and secrete several neurotrophic factors, adhesion molecules, and extracellular matrices.[45,46] En-

hanced polyol metabolism, which is exclusively found in Schwann cells,[47] might disturb this function of these cells and thereby impair nerve regeneration. The deleterious effect of increased polyol metabolism on nerve regeneration has been demonstrated in animal models with galactose intoxication, in which nerve CNTF was found to be decreased[42] and nerve regeneration across a silicone tube gap was delayed.[48]

The present study demonstrates that impaired nerve regeneration in diabetes may be associated with increased polyol metabolism and may contribute to the progressive nerve fiber loss in diabetic neuropathy. Since this impairment can be treated with tolrestat, ARIs may be beneficial even in advanced diabetic neuropathy in which considerable nerve fiber loss has occurred.

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