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Effects of cilostazol, an antiplatelet agent, on axonal regeneration following nerve injury in diabetic rats

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Abstract

To evaluate the ability of cilostazol, an antiplatelet and vasodilating agent, to promote axonal regeneration in streptozotocin-induced diabetic rats, the time until beginning of regeneration (initial delay) and the axonal regeneration rate of the sciatic nerve were estimated using the pinch test, and ornithine decarboxylase activity was measured in dorsal root ganglia. At 5 weeks of diabetes, axonal regeneration rate remained unchanged but the initial delay was prolonged and ornithine decarboxylase induction was delayed in diabetic rats compared with those in normal rats. Cilostazol had little effect on these parameters in normal or diabetic rats. At 10 weeks of diabetes, diabetic rats showed both prolongation of initial delay and a decrease in axonal regeneration rate. Cilostazol markedly increased axonal regeneration rate in diabetic rats. Ornithine decarboxylase induction following nerve injury disappeared almost completely in diabetic rats but was maintained by cilostazol treatment. The effect of cilostazol in diabetic rats is thought to be mediated through its preventive effect on circulatory disorders. The active site of the drug appears to be early processes in nerve regeneration before ornithine decarboxylase induction. Further, the results suggest that the both axonal regeneration and this induction are sensitive to circulatory defects in diabetes.

Keywords: Axonal regeneration; Ornithine decarboxylase; Streptozotocin diabetic rat; Nerve injury; Cilostazol; Antiplatelet vasodilating agent; Phosphodiesterase inhibitor

1. Introduction

Diabetic neuropathy, which is characterized by sensory and autonomic nerve dysfunction, is one of the most frequent complications in diabetic patients. Electrophysiological and morphological studies have reported delayed nerve conduction velocity, demyelination and axonal degeneration (Greenbaum et al., 1964; Dyck et al., 1986). It has also been suggested that nerve regeneration is not sufficient to compensate for the axonal degeneration in diabetic neuropathy (Longo et al., 1986; Bradley et al., 1995). The pathogenesis of diabetic neuropathy, which includes metabolic factors such as abnormal polyol metabolism, glycation and advanced glycation endproduct production as well as vascular factors has been reviewed by Yagihashi (1995). In experimental diabetic animals, various agents such as aldose reductase inhibitors, vasodilators, an inhibitor of advanced glycation endproduct, radical scavengers, gangliosides, Ca^{2+} channel antagonists and carnitine derivatives have all been reported to ameliorate or prevent the electrophysiological and morphological disorders in peripheral nerves. Although aldose reductase inhibitors such as ponalrestat and tolrestat have been studied with respect to their ability to promote nerve regeneration (Ekström and Tomlinson, 1989; Sima et al., 1993; Kamijyo et al., 1996); there are no such reports concerning vasoactive agents.

Cilostazol, which inhibits type III phosphodiesterase, is an antiplatelet agent with a vasodilating action (Kimura et al., 1985; Tanaka et al., 1988) and is currently used in Japan for the treatment of insufficient peripheral circulation (Okuda et al., 1992). A recent study of the drug using streptozotocin-induced diabetic rats, a model for insulin-dependent diabetes mellitus, showed that the drug increased the nerve blood flow (Kihara et al., 1995) and was effective to improve delayed nerve conduction velocity, decreased nerve Na^+,K^+-ATPase activity, axonal atrophy (Shindo et al., 1993; Uehara et al., 1997) and reduced axonal transport of cytoskeletal proteins (submitted).

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The present study examined nerve regeneration and ornithine decarboxylase induction, the latter being an essential event for the regeneration and survival of neurons (Myall et al., 1990), in the ganglion of normal and streptozotocin-induced diabetic rats in order to evaluate the ability of cilostazol to promote axonal elongation following cryolytic injury of the sciatic nerve and to characterize the action mechanism.

2. Materials and methods

2.1. Animals

Male Wistar rats (100–140 g, Nihon SLC, Japan) were used at 6 weeks of age. The animals were housed in a controlled environment (23 ± 2°C, 60 ± 10% relative humidity) and allowed free access to food and water. The room lights were off between 19:00 and 07:00 h. All experimental procedures were carried out in accordance with the guidelines and instructions for animal experimentation recommended by the Science Council of Japan.

2.2. Induction of diabetes

The animal were anesthetized with ether and made diabetic by an intravenous injection of streptozotocin (Wako) dissolved in 0.01 M citrate–saline buffer (pH 4.5) at a dose of 50 mg ml^{-1} kg^{-1}. Normal rats received 0.01 M citrate-saline buffer (pH 4.5) alone.

2.3. Experimental groups and administration of cilostazol

Two separate experiments were carried out; one was referred to as nerve regeneration and the other as ornithine decarboxylase induction. In both experiments, blood was collected from the tail vein 3 days after injection of streptozotocin and plasma glucose levels were determined using the glucose oxidase method (Glucose B-test Wako, Wako). Animals with a plasma glucose level of 400 mg/dl or higher were selected and assigned to either an untreated diabetic group or a cilostazol-treated diabetic group. Age-matched normal rats were assigned to either an untreated control group or a cilostazol-treated control group. Untreated controls and untreated diabetics were fed a commercial diet (MF, Oriental Yeast, Japan), and those in the cilostazol-treated control group and cilostazol-treated diabetic group were fed the same diet containing 0.03% cilostazol.

2.4. Nerve injury

Nerve injury was performed at 5 or 10 weeks after streptozotocin injection according to the procedure reported by Komiya (1980). The animal was anesthetized with ether and the sciatic nerve was exposed at the mid thigh level and frozen for 20 s by pressing it onto a 2 mm diameter copper wire that had been chilled in liquid nitrogen. The wound was closed and the animal was allowed to recover.

2.5. Regeneration distance of axon

The sciatic nerve on both sides of the animal was injured as described above. The rats were slightly anesthetized again with ether 3, 5 or 7 days following nerve injury and the sciatic nerve was re-exposed. The regeneration distance of the nerve was evaluated using a pinch test. The nerve was pinched with a pair of forceps, starting from 80 mm distal to the injury site and moving proximally in 0.5 mm steps until the typical muscle response was observed. The nerve was cut at this point (distal point). The animal was killed by cervical dislocation and the sciatic nerve was dissected out. The dissected nerve was cut again at the injury point under a microscope. The nerve length was measured and recorded as the regeneration distance of the axon. The value measured from both sciatic nerves was averaged and the value was defined as individual data. These procedures were carried out under a double blind procedure without knowledge of the specific treatment given to the animal.

2.6. Rate of axonal regeneration and initial delay

Regeneration distance was plotted as a function of time following nerve injury and the regression line was calculated by the least squares method. The rate of axonal regeneration was determined from the slope of the regression line. The initial delay, which represents the lag time before axons start regeneration, was obtained by extrapolation of the regression line to 0 mm regeneration.

2.7. Ornithine decarboxylase activity

In the ornithine decarboxylase experiment, the right sciatic nerve was injured as described above, and the left sciatic nerve was sham-operated. The wound was closed and the rats were allowed to recover for a period of 0, 1, 2 or 3 days before being killed by exsanguination under deep ether anesthesia. The L4 and L5 dorsal root ganglia were removed from each nerve and stored at -80°C. Ornithine decarboxylase activity was assayed as described previously (McLean et al., 1987) by trapping $^{14}CO_2$, which was liberated by conversion of ornithine to putrescine from [1-^{14}C]ornithine. The assay was performed as follows. Pairs of ganglia from each nerve were homogenized in 200 μl of 25 mM Tris buffer (pH 7.4) containing 1 mM EDTA, 5 mM dithiothreitol, and 0.1 mM pyridoxal-5′-phosphate. The homogenates were centrifuged at 100 000 $\times g$ for 30 min at 4°C and the supernatants were incubated for 2 h at 37°C with 50 mM Tris buffer (pH 7.4), 4 mM EDTA, 4 mM dithiothreitol, 0.4 mM pyridoxal-5′-phos-

Table 1
Effect of cilostazol on body weight, plasma glucose level, initial delay and regeneration rate in normal and diabetic rats 5 weeks after streptozotocin injection

Group	*n*	Body weight (g)	Plasma glucose level (mg/dl)	Axonal regeneration	
				Initial delay (day)	Regeneration rate (mm/day)
Untreated controls	(21)	300 ± 19	147 ± 11	0.35	3.19
Cilostazol-treated controls	(21)	300 ± 16	142 ± 13	0.58	3.38
Untreated diabetics	(21)	151 ± 23[a]	705 ± 113[a]	1.86[b]	3.44
Cilostazol-treated diabetics	(21)	143 ± 27[a]	736 ± 143[a]	1.63	3.56

Regeneration rate (mm/day; slope of regression line) and initial delay (day, *x*-axis intercept) were calculated by linear regression analysis of the regeneration distance as measured with the pinch test at 3, 5 and 7 days post injury.
Values are means ± S.D. (body weight and plasma glucose level).
[a] $P < 0.01$ compared with the untreated controls (one-way analysis of variance followed by two-tailed Tukey's *t*-test).
[b] $P < 0.01$ compared with the untreated controls (regression analysis).

phate and 0.2 μCi D, [1-^{14}C]ornithine (50 mCi/mmol, Amersham). The assay was performed in a 5-ml glass vial stoppered with rubber tubing containing 1 cm^2 of filter paper that was moistened with 40% KOH. The reaction was stopped by injecting 50 μl of 100% (w/v) trichloroacetic acid solution into the reaction mixture, and the mixture was allowed to stand for 2 h at room temperature. The filter paper and KOH solution were removed and the radioactivity was counted in 6 ml of liquid scintillation fluid (Atomlight, Amersham) using a liquid scintillation counter.

2.8. Statistics

All data are expressed in terms of means ± S.D. The axonal regeneration rate and initial delay were assessed by comparing two regression lines. Body weight, plasma glucose level and regeneration distance were assessed by comparing 4 groups in a one-way analysis of variance followed by a two-tailed Tukey's *t*-test. Comparisons of the response over time for ornithine decarboxylase following nerve injury were assessed by two-way analysis of variance followed by Tukey's *t*-test. Comparisons of ornithine decarboxylase activity between injured and uninjured sides were assessed by means of a two-tailed Student's paired *t*-test using values measured at each time point. Differences were considered to be statistically significant at the $P < 0.05$ level.

3. Results

3.1. Body weight and plasma glucose level

In all experiments, body weights were remarkably low and plasma glucose levels were notably higher in untreated diabetics than in the corresponding untreated controls (Tables 1–3). Cilostazol had little effect on body weights and plasma glucose levels in normal and diabetic rats. However, the plasma glucose level in the cilostazol-treated diabetic group was significantly lower (84% control, $P < 0.01$) compared to that in the untreated diabetics at 10 weeks after streptozotocin injection in the nerve regeneration experiment (Table 2), and body weight in the cilostazol-treated diabetic group was significantly higher (112% of control, $P < 0.05$) than that in the untreated diabetics at

Table 2
Effect of cilostazol on body weight, plasma glucose level, initial delay and regeneration rate in normal and diabetic rats 10 weeks after streptozotocin injection

Group	*n*	Body weight (g)	Plasma glucose level (mg/dl)	Axonal regeneration	
				Initial delay (day)	Regeneration rate (mm/day)
Untreated controls	(21)	351 ± 21	144 ± 7	1.15	3.81
Cilostazol-treated controls	(21)	350 ± 16	138 ± 4	1.10[c]	3.88
Untreated diabetics	(21)	155 ± 32[a]	810 ± 63[a]	1.79[c]	2.59[d]
Cilostazol-treated diabetics	(19)	162 ± 23[a]	680 ± 77[a, b]	1.84	3.83[e]

Regeneration rate (mm/day; slope of regression line) and initial delay (day, *x*-axis intercept) were calculated by linear regression analysis of the regeneration distance as measured with the pinch test at 3, 5 and 7 days post injury. Values are means ± S.D. (body weight and plasma glucose level).
[a] $P < 0.01$ compared with the untreated controls (one-way analysis of variance followed by two-tailed Tukey's *t*-test).
[b] $P < 0.01$ compared with the untreated diabetics (one-way analysis of variance followed by two-tailed Tukey's *t*-test).
[c] $P < 0.05$ compared with the untreated controls (regression analysis).
[d] $P < 0.01$ compared with the untreated controls (regression analysis).
[e] $P < 0.01$ compared with the untreated diabetics (regression analysis).

Table 3
Effect of cilostazol on body weight and plasma glucose level in the ornithine decarboxylase induction experiment

Group	5 weeks diabetes		10 weeks diabetes	
	Body weight (g)	Plasma glucose level (mg/dl))	Body weight (g)	Plasma glucose level (mg/dl)
Untreated controls	249 ± 19 (32)	115 ± 13 (32)	340 ± 13 (32)	140 ± 8 (32)
Cilostazol-treated controls	255 ± 14 (32)	100 ± 18 (32)	341 ± 16 (32)	134 ± 9 (32)
Untreated diabetics	162 ± 35 (32)[a]	709 ± 121 (32)[a]	161 ± 37 (31)[a]	744 ± 86 (31)[a]
Cilostazol-treated diabetics	165 ± 26 (31)[a]	629 ± 79 (31)[a]	181 ± 30 (32)[a,b]	721 ± 86 (32)[a]

Values are means ± S.D. (*n*).
[a] $P < 0.01$ compared with the untreated controls (one-way analysis of variance followed by two-tailed Tukey's *t*-test).
[b] $P < 0.05$ compared with the untreated diabetics (one-way analysis of variance followed by two-tailed Tukey's *t*-test).

10 weeks in the ornithine decarboxylase induction experiment (Table 3).

3.2. Axonal regeneration following nerve injury

3.2.1. Five weeks after streptozotocin injection

The linear regression analysis of regeneration distances, as measured by the pinch test at 5 weeks of diabetes, is shown in Fig. 1. No significant differences were observed in the rate of axonal regeneration between the untreated controls and the untreated diabetics (Table 1), meaning that the slopes of the regression lines were basically the same in these two groups. The initial delay in the untreated diabetics was prolonged compared with that in the untreated controls. Cilostazol had statistically no effect on the regeneration rate or the initial delay in normal and diabetic rats.

3.2.2. Ten weeks after streptozotocin injection

The regeneration rate in the untreated diabetics was significantly reduced compared to that in the untreated controls ($P < 0.01$, Fig. 2, Table 2). In the cilostazol-treated diabetic group, the regeneration rate increased significantly compared to that in the untreated controls and the value was similar to that in the untreated controls. The initial delay in the untreated diabetics was significantly prolonged ($P < 0.01$) compared with that in the untreated controls. Cilostazol had only a slight effect on the initial delay in normal rats but statistically no effect in diabetic rats.

3.3. Changes of ornithine decarboxylase induction following nerve injury

3.3.1. Five weeks after streptozotocin injection

The maximum increase in ornithine decarboxylase activity in the ganglia on the injured side could be detected 24 h following nerve injury in the untreated controls (Fig. 3). This increase was significant ($P < 0.01$) compared with that in the uninjured side, and the activity was more than 2-fold that of the uninjured side (uninjured: 1.12 ± 0.24 pmol h^{-1} $ganglia^{-1}$; injured: 2.58 ± 1.06 pmol h^{-1}

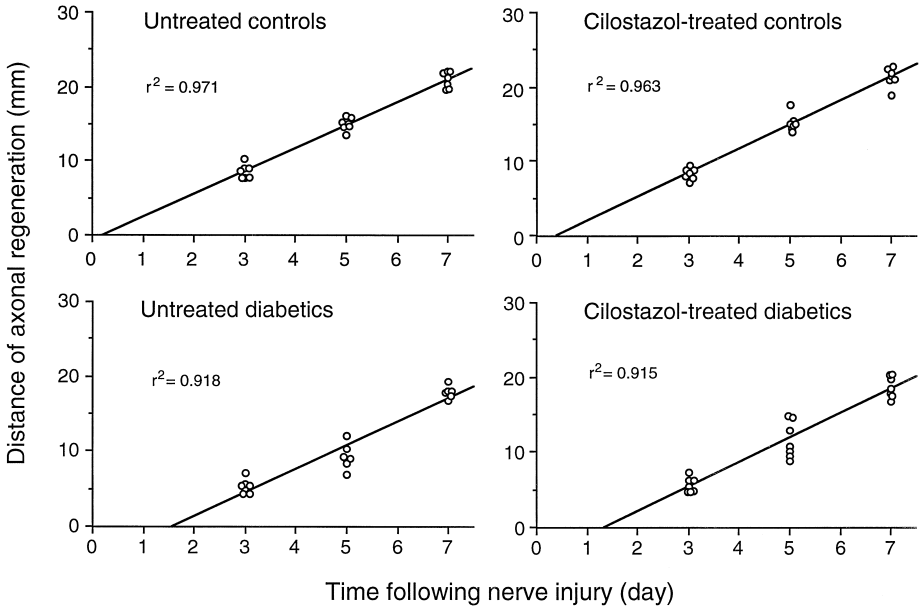

Fig. 1. Linear regression analysis of axonal regeneration in sciatic nerve following injury 5 weeks after streptozotocin injection. Distance of axonal regeneration in sciatic nerve measured by pinch test.

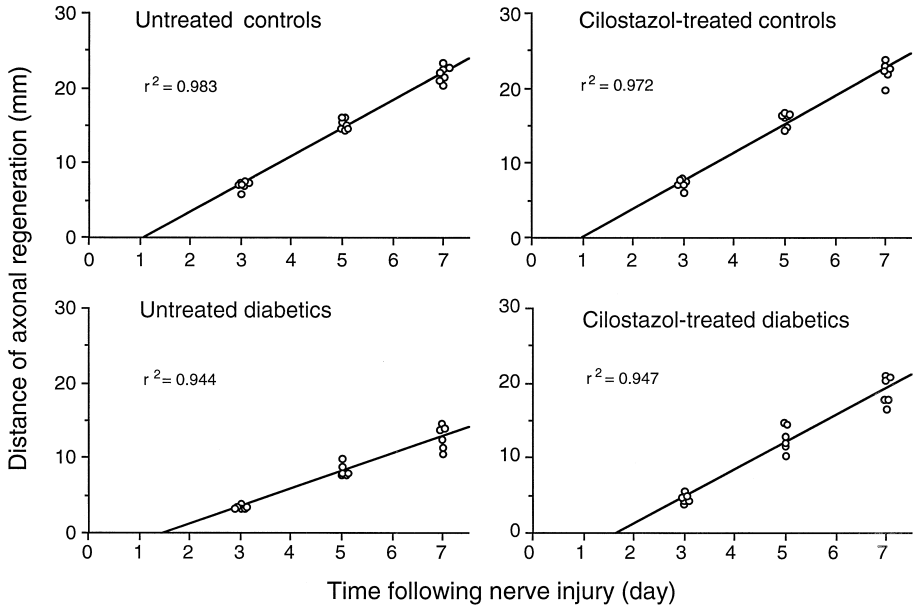

Fig. 2. Linear regression analysis of axonal regeneration in sciatic nerve following nerve injury 10 weeks after streptozotocin injection. Distance of axonal regeneration in sciatic nerve measured by pinch test.

ganglia^{-1}). A similar increase in ornithine decarboxylase induction was observed, in the cilostazol-treated control group. This activity increased significantly ($P < 0.05$, respectively) at 24 h (uninjured: 1.27 ± 0.41 pmol h^{-1} ganglia^{-1}; injured: 1.98 ± 0.82 pmol h^{-1} ganglia^{-1}) and 48 h (uninjured: 1.17 ± 0.20 pmol h^{-1} ganglia^{-1}; injured: 2.50 ± 0.73 pmol h^{-1} ganglia^{-1}) following injury. A significant increase in ornithine decarboxylase activity was also observed on the injured side ($P < 0.05$) compared to the uninjured side in the untreated diabetics (uninjured: 1.20 ± 0.17 pmol h^{-1} ganglia^{-1}; injured: 1.98 ± 0.99 pmol h^{-1} ganglia^{-1}) and in the cilostazol-treated diabetic group (uninjured: 1.11 ± 0.16 pmol h^{-1} ganglia^{-1}; injured: 2.23 ± 1.21 pmol h^{-1} ganglia^{-1}), but the activity peaks were retarded to 48 h.

3.3.2. Ten weeks after streptozotocin injection

As shown in Fig. 4, ornithine decarboxylase induction was observed to peak at 24 h following injury in both the untreated controls (uninjured: 1.40 ± 0.40 pmol h^{-1}

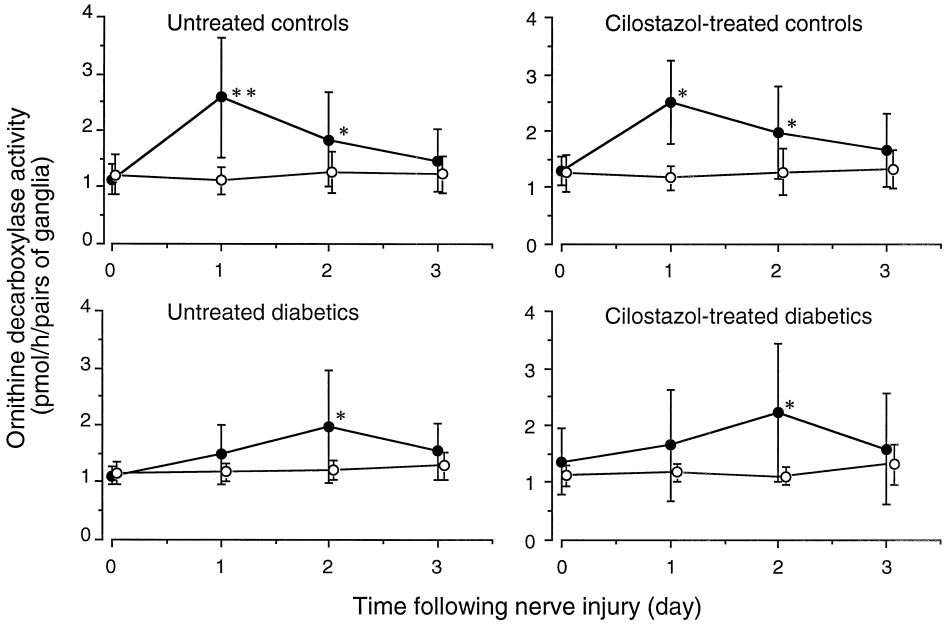

Fig. 3. Changes in ornithine decarboxylase activity in dorsal root ganglia (L4 and L5) following nerve injury at 5 weeks after streptozotocin injection. Ornithine decarboxylase activity in the ganglia on injured (●) and uninjured (○) sides measured at each time point following nerve injury. Values are means ± S.D. ($n = 8$). $*$ $P < 0.05$, $**$ $P < 0.01$ compared with corresponding uninjured side (two-tailed Student's paired t-test).

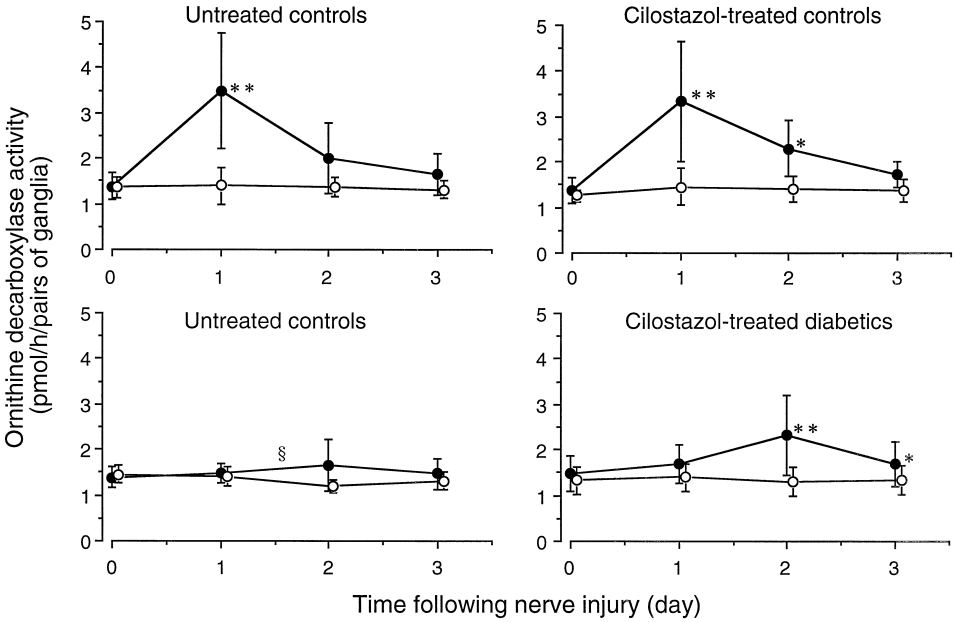

Fig. 4. Changes in ornithine decarboxylase activity in dorsal root ganglia (L4 and L5) following nerve injury at 10 weeks after streptozotocin injection. Ornithine decarboxylase activity in the ganglia on injured (●) and uninjured (○) sides measured at each time point following nerve injury. Values are means ± S.D. ($n = 7–8$). $*P < 0.05$, $**P < 0.01$ compared with corresponding uninjured side (two-tailed paired Student's *t*-test). $*P < 0.05$, $**P < 0.01$ compared with corresponding uninjured side (two-tailed paired Student's *t*-test). $^{§}P < 0.05$, significant differences in response over time following nerve injury compared with the untreated controls (two-way analysis of variance followed by two-tailed Tukey's *t*-test).

ganglia^{-1}; injured: 3.49 ± 1.27 pmol h^{-1} ganglia^{-1}) and cilostazol-treated control group (uninjured: 1.46 ± 0.40 pmol h^{-1} ganglia^{-1}; injured: 3.33 ± 1.31 pmol h^{-1} ganglia^{-1}). Ornithine decarboxylase was almost lost in the untreated diabetics (uninjured: 1.21 ± 0.14 pmol h^{-1} ganglia^{-1}; injured: 1.65 ± 0.58 pmol h^{-1} ganglia^{-1}) but was maintained in the cilostazol-treated diabetic group compared to the untreated diabetics (uninjured: 1.30 ± 0.33 pmol h^{-1} ganglia^{-1}; injured: 2.31 ± 0.88 pmol h^{-1} ganglia^{-1}) but the peak of ornithine decarboxylase induction was retarded to 48 h.

4. Discussion

The present results demonstrated that cilostazol, an antiplatelet agent that exerts a vasodilator effect, markedly prevented a decrease in the axonal regeneration rate following cryolytic injury to the sciatic nerve in streptozotocin-induced diabetic rats. This impairment of axonal regeneration in diabetic rats is consistent with a previous report (Ekström and Tomlinson, 1989) in which the axonal regeneration distance was shortened in diabetic rats with 5 weeks' diabetes. Ornithine decarboxylase induction following nerve injury is thought to be closely related to the cell body response and subsequent nerve repair including RNA metabolism (Russell, 1983) and protein synthesis (Gilad and Gilad, 1983). Our observation that ornithine decarboxylase is induced in the ganglia following sciatic nerve injury in normal rats but is impaired in diabetic rats agrees with a previous report (McLean et al., 1987). The present experiment revealed that the initial delay and regeneration rate are correlated with the time course of ornithine decarboxylase induction and degree of ornithine decarboxylase induction, respectively.

Cilostazol is an antiplatelet and vasodilating agent that inhibits type III phosphodiesterase and increases the cyclic AMP content in platelets (Tani et al., 1992) and blood vessels (Tanaka et al., 1988). Theophylline, dibutyryl cyclic AMP and forskolin decreased the initial delay following crush injury to the sciatic nerve in normal rats (Kilmer and Carlsen, 1987). Although cilostazol shows little effect on the initial delay in normal and diabetic rats, it has been shown to inhibit the decrease in the axonal regeneration rate in the diabetic rat, suggesting that the mechanism of cilostazol action differs from that of cyclic AMP mimetic agents. In the present experiments, cilostazol also unexpectedly lowered the plasma glucose level and increased body weight in diabetic rats. However, because these changes were only −16% and +12%, the improvement in hyperglycemia and body weight loss did not likely contribute greatly to the amelioration of nerve regeneration disorders. These results, together with the fact that cilostazol increases nerve blood flow in diabetic rats (Kihara et al., 1995), led us to speculate that the drug secondarily prevented a decrease in the axonal regeneration rate by ameliorating complications associated with diabetes mellitus such as ischemia and hypoxia.

In the present study, cilostazol exerted little or no effect on nerve regeneration and initial delay in normal rats. This

may be because the intake of cilostazol was lower in normal rats than in diabetic rats as suggested by the results of our previous study in which we measured the food intake of rats using the same diabetic model. The cilostazol intake was 46 and 44 mg g^{-1} b.wt day^{-1} in normal rats, and 164 and 149 mg g^{-1} b.wt day^{-1} in diabetic rats at 5 and 9 weeks of diabetes, respectively. We also found that cilostazol did not influence food intake in either normal or diabetic rats.

Axonal regeneration following nerve injury is known to proceed as follows: neurogenic inflammation and the invasion of macrophages occurs very early in this process (Perry et al., 1987; Dahlin, 1995). This is followed by Schwann cell proliferation, at which time neurotrophic factors are released and specific injury signals are transported to the nerve cell bodies (Kanje et al., 1986; Myall et al., 1990). Ornithine decarboxylase is induced and proteins necessary for nerve regeneration are synthesized in the cell bodies. Newly synthesized and reconstituted cytoskeletal proteins are then transported to the regenerating site (Tashiro and Komiya, 1991). In diabetes, some of these processes are impaired by an abnormal polyol metabolism, glycation and tissue hypoxia as well as by circulatory dysfunction. The close correlation between nerve regeneration and ornithine decarboxylase induction, as well as impairment found in both in the present study and in other studies (McLean et al., 1987; Ekström and Tomlinson, 1989) suggest that the lower axonal regeneration rate is due to impairment at various stages from the initial inflammation at the injury site to ornithine decarboxylase induction in the cell bodies. Neurogenic flare (Gamse and Jancso, 1985; Zochodne and Ho, 1993), and retrograde axonal transport (Schmidt et al., 1983) are both reportedly impaired in diabetic rats.

Although the factors that stimulate ornithine decarboxylase activity in response to nerve injury are not yet understood, in this experiment, ornithine decarboxylase activity of the ganglia from uninjured nerve did not differ between the normal and diabetic rats, suggesting that the constitutive activity of ornithine decarboxylase was retained in diabetic rats. Furthermore, many morphometric studies have shown no significant fiber loss in the major nerve trunks of streptozotocin-induced diabetic rats (Sharma and Thomas, 1974; Wright and Nukada, 1994). This, combined with the present data, suggests that little or no degeneration of the sciatic nerve occurs in short-duration diabetic rats. Furthermore, Kanje et al. (1986) reported that injection of vinblastin, an inhibitor of retrograde and orthograde axonal transport, into the nerve inhibited ornithine decarboxylase induction in the ganglia following nerve crush. The surgical procedure itself did not appear to have affected ornithine decarboxylase activity in the ganglia of uninjured side in the present study. Thus, signals for ornithine decarboxylase induction appear to be derived from the site of injury in the nerve and are then transported retrogradely to the ganglion.

In addition, an aldose reductase inhibitor (Pekiner and McLean, 1990) and cilostazol reportedly prevent impairment of ornithine decarboxylase induction in diabetic rats, suggesting that the these processes are sensitive to both abnormal polyol metabolism and circulatory disorders.

Alternatively, the decrease in axonal regeneration could be explained by an impaired protein synthesis in the cell bodies following ornithine decarboxylase induction. Amino acid uptake, which is necessary for protein synthesis, was reduced in the ganglia of diabetic rats (Thomas et al., 1984). Although it is not yet known whether cilostazol increases amino acid uptake and protein synthesis, the drug does increase Na^+,K^+-ATPase activity (Uehara et al., 1997), which plays an important role in amino acid uptake (Greene et al., 1990) in peripheral nerves of diabetic rats. In addition to protein synthesis, anterograde axonal transport of cytoskeletal proteins reportedly decreases in diabetic rats (Sidenius and Jakobsen, 1979), and this may be another important factor in nerve regeneration following nerve injury. Cilostazol might thus prevent a decrease in the axonal regeneration rate in diabetic rats mediated by its ameliorating effect on reduced anterograde axonal transport rate of cytoskeletal proteins (submitted).

The mechanism of cilostazol action, other than its effects on circulatory disorders, can be determined by its effect on macrophages, Schwann cells and neurons. Macrophages contain type III phosphodiesterase (Tenor et al., 1995) which is inhibited by cilostazol, but an elevated cyclic AMP also inhibits the function of macrophages (Zhong et al., 1995). It is not yet known whether type III phosphodiesterase exists in the nervous system. Therefore, the action of cilostazol in axonal regeneration appears to be due mainly to its circulatory effects.

The present study examined the effect of cilostazol on short-term regeneration following nerve injury in rats treated with cilostazol immediately after the induction of diabetes. Clinically, long-term repair and nerve maturation including remyelination are also important. Although further morphological studies are necessary to elucidate the effect of cilostazol on long-term regeneration, the marked inhibition of cilostazol on early impairment of axonal regeneration suggests its efficacy in the long-term repair and regeneration process.

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