Cervical spondylotic myelopathy is a common condition that progressively impairs motor and sensory functions. The disturbances result from chronic cervical spinal cord compression, caused by degenerative changes in the vertebrae, such as osteophyte formation, disc protrusion, hypertrophy or ossification of ligaments, and slippage of the vertebrae. Neuronal loss and vacuolar degeneration have been observed in the spinal cord of autopsied cases and several experimental animal models. It has been postulated that local spinal cord circulatory insufficiency due to the compression plays a significant role in the pathogenesis and progression of the disorder.

Effective nonsurgical treatments for cervical spondylotic myelopathy have not been established. Surgical decompression of the cervical cord is warranted for improving and preventing neurological deterioration; however, especially in the elderly, systemic comorbidities often preclude this treatment strategy. For amelioration of symptoms and prevention of disease progression in such patients, an effective pharmacological treatment is desirable.

Cilostazol, a selective Type III phosphodiesterase inhibitor: prevention of cervical myelopathy in a rat chronic compression model

Laboratory investigation


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Object. Regional blood flow is decreased in experimental models of chronic spinal cord compression, and the alteration presumably contributes to the development of myelopathy. Cilostazol (Otsuka Pharmaceuticals Co.), a selective Type III phosphodiesterase inhibitor, has been shown to be neuroprotective in cerebral hypoperfusion animal models and clinically effective in preventing the recurrence of cerebral infarction. To investigate the neuroprotective effect of cilostazol on cervical spondylotic myelopathy, the preventive effect against progressive motor dysfunction and the loss of anterior horn motor neurons were assessed using a chronic cord compression model in rats.

Methods. To produce chronic cervical cord compression in male Wistar rats, thin polyurethane sheets (3 × 5 × 0.7 mm) that gradually expand over 48–72 hours by absorbing water were implanted under the C5–6 laminae. In sham operations, the sheets were momentarily placed and then immediately removed. This model has been shown to reproduce characteristic features of clinical cervical myelopathy, with progressive motor disturbances after a latency period and insidious neuronal loss preceding the onset of symptoms. In the treatment group, cilostazol (30 mg/kg/day) was orally administered to the rats once a day, starting the day after surgery and continuing through the entire observation period of 25 weeks. In the control group, vehicle solution was administered under the same protocol. Changes in motor function were monitored by measuring bilateral forepaw grip strength and the duration of forced running on a treadmill. Twenty-five weeks after surgery, cervical spinal cords were examined histopathologically.

Results. Cilostazol preserved both forepaw grip strength and forced running capability. The drug also preserved anterior horn motor neurons in the C5–6 spinal cord segment, which diminished in number in the untreated chronic compression group. The drug decreased the number of TUNEL-positive apoptotic cells.

Conclusions. These results indicate that cilostazol is neuroprotective in the chronically compressed cervical cord and is potentially useful in the treatment of cervical spondylotic myelopathy.

KEY WORDS • cervical myelopathy • cervical spondylosis • cilostazol • motor function • motor neuron • apoptosis • trauma • rat

Cervical spondylotic myelopathy is a common condition that progressively impairs motor and sensory functions. The disturbances result from chronic cervical spinal cord compression, caused by degenerative changes in the vertebrae, such as osteophyte formation, disc protrusion, hypertrophy or ossification of ligaments, and slippage of the vertebrae. Neuronal loss and vacuolar degeneration have been observed in the spinal cord of autopsied cases and several experimental animal models. It has been postulated that local spinal cord circulatory insufficiency due to the compression plays a significant role in the pathogenesis and progression of the disorder.

Effective nonsurgical treatments for cervical spondylotic myelopathy have not been established. Surgical decompression of the cervical cord is warranted for improving and preventing neurological deterioration; however, especially in the elderly, systemic comorbidities often preclude this treatment strategy. For amelioration of symptoms and prevention of disease progression in such patients, an effective pharmacological treatment is desirable.

This article contains some figures that are displayed in color online but in black-and-white in the print edition.
Cilostazol (6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydro-2-(1H)-quinolinone), a potent inhibitor of Type III phosphodiesterase, has been approved for the treatment of intermittent claudication by the FDA in the US and for the prevention of recurrent ischemic stroke in Asian countries including Japan, Korea, Hong Kong, and China. Its principal actions are inhibition of platelet aggregation and vasodilation by increasing the cyclic adenosine monophosphate (cAMP) level. Recent clinical trials using a randomized placebo-controlled and aspirin-controlled double-blind method showed that long-term administration of cilostazol is safe and effective in preventing a recurrence of cerebral infarction. Some reports described other effects, such as antioxidant and antiapoptotic, which may ameliorate neuronal damage in experimental chronic cerebral ischemia.

In this study, we investigated the neuroprotective effects of cilostazol in an established experimental model of chronic cervical cord compression by evaluating the alteration in motor function and histopathology.

**Methods**

**Animals**

Animal care and experimental protocols complied with the US Public Health Service Policy on Humane Care and Use of Laboratory Animals and were approved by the Animal Welfare Committee of Dokkyo University School of Medicine. Forty male Wistar rats (Japan SLC Inc.; maximum resolution 72 hours to reach 230%), ages 12–14 weeks and weighing 250–270 g, were used. The animals were maintained in a specific pathogen-free environment, with air conditioning maintained at 23°C and ambient light controlled diurnally. The rats were kept in cages with an attached revolving wheel (SN451, Shinano Manufacturing) for quantitative evaluation of voluntary exercise and were allowed free access to water and food. Body weight was recorded weekly. Before surgery, they were kept in the cages for 3 weeks for adaptation to the environment and the exercise.

**Chronic Cervical Cord Compression Model**

The method to induce chronic compression in the cervical cord has been described in detail. After inducing anesthesia with pentobarbital (25 mg/kg, intraperitoneal injection) and 1% isoflurane in a chamber, the animals were maintained on spontaneous ventilation with 0.5%–1.0% isoflurane administered through a facial mask. Rectal temperature was maintained at 37°C–37.5°C using a thermistor-controlled heating pad. A midline incision was made in the nuchal area, and the C4–C6 laminae were exposed. The yellow ligament between the laminae was removed. The dura mater underneath was carefully separated from the laminae to avoid a tear and CSF leak. A sheet of expanding urethane-compound polymer (Aquaprene C, Sanyo Chemical Industries), measuring 3 × 5 × 0.7 mm, was inserted in the C5–6 sublaminal space using a surgical microscope. The size of the sheet was measured with a micrometer and adjusted precisely. The urethane polymer sheet expands after implantation, absorbing tissue water over 48–72 hours to reach 230% in volume; thereafter, the volume remains constant. This polymer sheet induces no evident tissue reaction, such as inflammation or granulation, surrounding the implant. In the animals allocated to the compression group, the polymer sheet was left in place and the wound was closed. In the animals allocated to the control (sham-operation) group, the polymer sheet was placed underneath the laminae momentarily and then removed. After recovery on a heating pad, the rats were returned to the wheel cage. This animal model properly reproduces characteristic features of clinical cervical myelopathy, with progressive motor disturbance after a delayed onset. In the compressed segment of the cord, the number of anterior horn motor neurons diminishes progressively. Twenty-five weeks after the operation, sinusoidal dilation of veins and small caviations of the gray matter are prominent. Although some demyelination of both gray and white matter is evident, inflammatory features are absent.

**Experimental Design and Groups**

To investigate the efficacy of cilostazol in preventing the development of symptomatic myelopathy, drug treatment was started on the day after surgery. Cilostazol (Otsuka Pharmaceuticals Co.) was suspended in 0.5% carboxymethyl cellulose sodium salt (Wako Pure Chemical Industries) to a concentration of 7.5 mg/ml. Animals in the drug treatment groups received 4 ml/kg of this solution (thus, 30 mg/kg/day of cilostazol) orally once every day. Animals in the vehicle control groups received the same amount of 0.5% carboxymethyl cellulose sodium salt solution orally on the same schedule. The dose of 30 mg/kg/day was chosen since it corresponds to the clinical dose used for peripheral arterial disease and cerebral stroke. Forty rats were allocated to the following 4 groups: Group A, the rats underwent a sham operation and received vehicle solution (sham operation + vehicle, n = 7 rats); Group B, the rats underwent sham operation and received cilostazol (sham operation + cilostazol, n = 7 rats); Group C, the rats underwent polymer sheet implantation and received vehicle solution (compression + vehicle, n = 13 rats); and Group D, the rats underwent polymer sheet implantation and received cilostazol (compression + cilostazol, n = 13 rats).

**Evaluations of Motor Function**

**Voluntary Exercise.** Rotations of the wheel attached to the cage were cumulatively recorded in either direction using an odometer. The count was recorded every week. The amount of voluntary exercise after surgery was expressed as a percentage of the average recorded during the 2-week period immediately before the surgery.

**Forepaw Grip Strength.** Forepaw grip strength was measured according to the methods described by Meyer et al. and Anderson et al. Rats were gently held with their hindlimbs supported and one forelimb restrained. The forepaw being tested was made to hold the handle of the digital force meter (FGP-0.5, Nippondensan Simpo Inc.; maximum resolution ± 0.1 g). The rats were allowed about 1 second to establish a grip and then were gently
Pulled back in one smooth motion until the grip was released. Measurement of the grip strength was considered valid when the rat grasped the bar immediately with all fingers and, on release, the paw was relaxed and not clenched. The measurement was not scored if the bar was hooked by a claw of spastic clenched paw. The strength value was defined as the maximum force recorded on the digital force gauge before the rat released the bar. Testing stopped after 5 legitimate scores were recorded for each paw. The handling and training took 3 weeks to complete, after which surgery was performed. Thereafter, grip strength was tested (5 trials/paw/session) twice per week for 25 weeks. The forepaw grip strength, measured in 10 trials a week, was averaged and expressed as a percentage of the average measured during the 2-week period immediately before surgery.

**Forced Locomotion Capability.** Before surgery, the rats underwent several sessions of forced exercise on a rotating treadmill (Rotor Road SN-445, Shinano Manufacturing). They were made to run on the rotor, 90 mm in diameter and covered with friction rubber. A timer coupled with a magnetic switch was stopped when the rats fell from the rotor, and the duration of the exercise was recorded. In a series of preliminary experiments using myelopathic rats, we determined that the optimal speed for detection of locomotor disturbance was 10 rpm.20 After the preliminary session, most of the animals were able to walk on the rotating treadmill continuously for more than 300 seconds, and animals that could reproducibly meet this condition were used for the experiments. The animals with chronic cord compression, in contrast, could not endure the exercise for this period of time, and thus animals with chronic cord compression, in contrast, could not endure the exercise for this period of time, and thus could not meet this condition were used for the experiments. One anatomical segment of the cervical cord is roughly 3 mm long, and our previous study revealed that the range covered by the 300 sections (3.0 mm) was sufficient to cover fluctuations in the motor neuron density along the axis.20

To assess axonal degeneration, specimens were stained with Luxol fast blue dye. To evaluate alterations in spinal cord contour (flattening) at the level of maximal compression, we calculated the flattening ratio of spinal cords: (minor axis/major axis) × 100.

**Terminal Deoxynucleotidyl Transferase (TdT)–Mediated Deoxyuridine Triphosphate (dUTP)–Biotin In Situ TUNEL Staining.** For the detection of cells undergoing apoptosis, TdT-mediated dUTP-biotin in situ TUNEL staining was performed using a commercially available kit (ApopTag Plus In Situ Apoptosis Detection kit, Chemicon International) according to the manufacturer’s instructions. The TUNEL-positive cells containing apoptotic bodies and condensed nuclear fragments were identified as apoptotic cells.11 After deparaffinization and hydration, tissue sections were washed in Tris-buffered saline, and permeabilized using proteinase K (20 μg/ml for 10 minutes), followed by 5 minutes of quenching in 3% H2O2 in methanol at room temperature. The sections were incubated in equilibration buffer for 20 minutes before labeling for 60 minutes at 37°C, and the reaction with TdT was stopped by the addition of stop buffer. After being washed in Tris-buffered saline, the sections were counterstained with hematoxylin and embedded in paraffin.

**Histological Evaluations**

**Tissue Preparation.** Twenty-five weeks after surgery, the rats were anesthetized with pentobarbital (25 mg/kg, intraperitoneally) and 1% isoflurane administered through a facial mask. Transcardial perfusion was performed with 200 ml of 0.01-M phosphate-buffered saline and then with 200 ml of 4% paraformaldehyde in 0.1-M phosphate buffer (pH 7.4, 4°C). The cervical spine was removed en bloc and placed in 4% paraformaldehyde solution for 2 days. After that, the spinal cord was dissected from the vertebrae and dura under a microscope and was embedded in paraffin.

**Cord Flattening and Motor Neuron Count.** The C5–6 segment of the cord was sectioned serially with a slice thickness of 5 μm and a gap interval of 5 μm over a 3000-μm length, with the midpoint at the interspace between the C-5 and C-6 vertebral bodies. Three hundred serial sections were stained with H & E, and the motor neurons in the anterior horn gray matter were counted. The rationale for the authenticity of the neuron count is described elsewhere.1,20,21 To obtain a precise count of the neurons without redundancy or omission, we chose a slice thickness of 5 μm with a gap interval of 5 μm, based on the following stereological considerations. Motor neurons were identified by the presence of their large nuclei and well-developed, densely stained Nissl bodies in the cytoplasm. The nucleus, normally centrally placed, contains a well-demarcated round nucleolus.34 The characteristic large nucleoli have a uniform diameter of approximately 5 μm (Fig. 1). With a slice thickness and interval identical to the diameter (5 μm) of the spherical nucleolus, each section contains tangential contours of the nucleoli with their centers located within 2.5 μm outside the slice. Thus, counting nucleoli of the motor neuron that appears on a 5-μm slice yields the number of those with the center located within 5 μm on each side from the middle of the slice. The 5-μm intervals between the sections means the stereological count avoids omission or redundancy in the number of motor neurons. One anatomical segment of the cervical cord is roughly 3 mm long, and our previous study revealed that the range covered by the 300 sections (3.0 mm) was sufficient to cover fluctuations in the motor neuron density along the axis.20

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incubated in anti-digoxigenin peroxidase for 30 minutes at room temperature and reacted with diaminobenzidine solution. The sections were counterstained with hematoxylin. The number of dark gray–colored TUNEL-positive cells was counted in 12 randomly chosen cross-sections with a thickness of 5 μm and a gap interval of more than 60 μm to avoid redundancy in the counts.

**Statistical Analysis**

Data are expressed as the means ± standard error of the mean. Body weight, the ratio of wheel rotation, the ratio of bilateral forepaw grip strength, and the time of forced exercise were tested using repeated measures ANOVA. When a significant difference among the groups was detected, individual sets of data were further compared using 1-way ANOVA. The cord flattening ratio and the number of cell counts were assessed using 1-way ANOVA. A p < 0.05 was considered statistically significant. Statistical analysis was performed using the JMP7 software package (SAS Institute Inc.).

**Results**

**Body Weight and Voluntary Exercise Activity**

The body weight of the animals in all groups decreased for 1 week after surgery. Thereafter, body weight increased gradually in all groups without significant intergroup differences. After surgery, voluntary exercise activity, as measured by the cumulative count of the revolving wheel, decreased transiently for 1 week (64.4% ± 13.1%, 59.4% ± 7.7%, 42.9% ± 6.5%, and 47.1% ± 10.2% in Groups A, B, C, and D, respectively; p = 0.39) and then recovered in 2 weeks (102.4% ± 10.9%, 102.0% ± 8.9%, 96.7% ± 5.4%, and 96.7% ± 11.3% in Groups A, B, C, and D, respectively; p = 0.96). During the 25 weeks after surgery, the wheel count decreased gradually without significant intergroup differences.

**Forepaw Grip Strength**

In the sham-operation groups (Groups A and B), bilateral forepaw grip strength did not change during the 25-week period. In the compression + vehicle group (Group C), bilateral grip strength deteriorated after surgery and recovered in several weeks. Progressive weakness started after a latency period of 6 weeks (Fig. 2). A significant difference in strength was observed at 7 weeks and thereafter in the right forepaw and at 8 weeks and thereafter in the left forepaw (p < 0.05). In the group of animals with cord compression + cilostazol (Group D), bilateral forepaw grip strength also deteriorated transiently after surgery but was preserved thereafter throughout the 25-week period.

**Forced Locomotion Capability**

Forced locomotion capability, measured as the duration of running on a rotating treadmill (cutoff at 300 seconds), remained fairly constant in the animals of Groups A and B. It deteriorated progressively in the animals with cord compression + vehicle (Group C), with a latency period of 18 weeks (Fig. 3). Comparison of the corresponding sets of data between Groups C and A at each time point showed significant differences after 19 weeks (p < 0.05). In the group with cord compression + cilostazol (Group D), no decrease in locomotion was observed.

**Cord Flattening and Loss of Anterior Horn Motor Neurons**

The flattening ratio at the C5–6 spinal cord was 58.2% ± 3.7%, 58.4% ± 3.2%, 43.5% ± 1.3%, and 43.4% ± 2.2% in Groups A, B, C, and D, respectively. The difference was significant between Groups A and C but not between Groups C and D (Figs. 4 and 5). In the group of animals with cord compression (Groups C and D), sinusoidal dilation of veins and small cavitations of the gray matter were observed. Cavity formation and myelin ovoids were also observed in the anterior, lateral, and posterior columns. Demyelination was not remarkable, and inflammatory changes were not evident in all groups. The number of motor neurons in the C5–6 anterior horns for the 3000-μm segment was 2591 ± 91.5 for Group A animals and 2664 ± 64.2 for Group B animals. The count was 1699 ± 60.4 in Group C animals (compression + vehicle), a 34.4% loss compared with the value in Group A. In the Group D animals (compression + cilostazol), the count was 2408 ± 76.5, a 7.1% loss compared with the value in Group A. A significant difference was detected between Groups C and D (p < 0.05; Fig. 6).

**TUNEL-Positive Cell Counts**

The average number of TUNEL-positive cells in the 12 randomly chosen slides was separately counted in 5 areas: anterior horn, posterior horn, anterior column, lateral column, and posterior column. The TUNEL-positive cells were not detected in the sham-operated animals (Groups A and B). In the animals with cord compression + vehicle (Group C), TUNEL-positive cells were present, particularly in the white matter (Figs. 7 and 8). In comparison, the number of TUNEL-positive cells in the animals with cord compression + cilostazol (Group D) was significantly lower in the gray and white matter.

**Discussion**

This study revealed that oral administration of cilostazol in a clinical dose provides significant protection against the development of myelopathy in the setting of chronic compression to the cervical cord. With this drug, motor function and anterior horn motor neurons were preserved. To the best of our knowledge, this report is the first to demonstrate that early intervention with cilostazol can prevent loss of motor neurons in a chronic spinal cord compression model.

It has been postulated that local spinal cord circulatory insufficiency plays a significant role in the pathophysiology of cervical spondylotic myelopathy, causing loss of motor neurons and vacuolar degeneration in the gray matter, along with various degrees of demyelination and axonal swelling in the white matter. 2,12–14,16,20,22,23,25 In the clinical situation of spondylotic myelopathy, despite preexisting cord impingements, motor weakness and
sensory disturbance start to be noticeable after a latency period and progress thereafter. This animal model reproduces characteristic features of delayed progressive motor dysfunction after a latency period as well as insidious neuronal loss preceding the onset of symptoms. We previously demonstrated a decrease in perfusion under mechanical cord compression in this model by using a fluorescent microsphere method. In the present study, the forced running capability of the rats on a rotating treadmill started to progressively deteriorate 18 weeks after the operation. To assess neurological dysfunction, which would be correlated to the compressed segment of C5–6, we measured forepaw grip strength. Deterioration became apparent at 7–8 weeks after induction of cord compression, and the changes were prevented by the oral administration of cilostazol. Histologically, the motor neurons in the segments were preserved by the treatment.

Cilostazol inhibits Type III phosphodiesterase, increases cyclic adenosine monophosphate (cAMP) concentrations, and consequently inhibits platelet aggregation. It also exhibits vasodilatory activity, inhibits vascular smooth muscle proliferation, and protects the vascular wall and en-
dothelium in vivo and in vitro. In several randomized trials, cilostazol significantly improved symptoms of intermittent claudication in patients with peripheral artery disease by improving blood flow and inhibition of platelet aggregation. The Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease (TASC) II international guidelines recommend cilostazol as the first-line treatment for intermittent vascular claudication. In the Cilostazol Stroke Prevention Study (CSPS) I and II, cilostazol significantly lowered the incidence of recurrent cerebral infarction without increasing the occurrence of cerebral hemorrhage. Cilostazol is also effective in preventing secondary cerebral infarction, particularly in patients with lacunar infarction and in high-risk patients with diabetes or hypertension. These results suggest that cilostazol protects against small-vessel disease and atherosclerosis. Based on this evidence, cilostazol has been used in many Asian countries for the prevention of secondary cerebral infarction and is listed in the guidelines for the management of stroke.

Previous reports showed that cilostazol improved blood flow in cerebral hypoperfusion models and reduced volume of infarction. Lee et al. demonstrated that cilostazol (30 mg/kg, orally twice) reduced infarct volume and intensity of cytotoxic edema with associated neurologic improvement in rats subjected to 2-hour occlusions of the middle cerebral artery (MCA) followed by 24-hour reperfections. Honda et al. showed that cilostazol attenuated gray and white matter damage with improvement in regional cerebral blood volume and cerebral blood flow in rats at 24 hours after MCA occlusion. In addition to its circulatory effect, cilostazol has neuroprotective properties ascribed to its antioxidative, antiinflammatory, and antiapoptotic actions. It suppresses microglial activation and reduces the level of cytotoxic substances, including inflammatory cytokines such as tumor necrosis factor (TNF-α). Watanabe et al. demonstrated that continuous administration of cilostazol improved learning memory, suppressed the early accumulation of lipid peroxidation products, diminished sequential inflammatory responses, and decreased the number of apoptotic cells in a rat chronic cerebral hypoperfusion model. Choi et al. demonstrated that cilostazol (10 mg/kg, intravenously) reduced the volume of cerebral infarcts, inhibited DNA fragmentation in association with increased Bcl-2 protein, and decreased Bax protein and cytochrome c release in the rat MCA occlusion and reperfusion model. Using the same model, Lee et al. demonstrated that cilostazol (30 mg/kg, orally twice) reduced infarct volume with improvement in neurological function, decreased apoptotic neuronal death, and reduced myeloperoxidase activity, which is a marker of neutrophil infiltration, in the penumbral region. They also demonstrated that cilostazol significantly reduced apoptosis in association with decreased TNF-α production and the number of cells posi-

**Fig. 4.** Alterations of the cord contour in each group: ratio of the sagittal (ventrodorsal) to the transverse width of the cord (flattening ratio) was measured on cross-sections at the C5–6 spinal cord in the 25 weeks after the operation. Marked indentations were observed in Groups C and D. Asterisks denote significant differences between groups (p < 0.05). comp = compression; ns = not significant.

**Fig. 5.** Photomicrographs (A–C) of the cervical cord section at C5–6 in the rats 25 weeks after the operation. H & E staining. Insets in panels A–C are magnified in panels D–F, respectively. In Group C, a decrease in the number of motor neurons was evident (E). In Group D, motor neurons in the anterior horn were preserved (F). Bars = 1000 μm (A–C) and 200 μm (D–F).
Cilostazol in preventing development of cervical myelopathy in rats

tive for caspase-3, consequently ameliorating vacuolation and rarefaction in the cerebral white matter of the chronic hypoperfusion model. These pleiotropic effects may contribute to the neuroprotective effect of cilostazol in the compression of the cervical spinal cord.

Sekiguchi et al. reported that cilostazol improved the blood flow and nerve conduction velocity of cauda equina in a short-term mechanical compression model in canines. Our study is unique in demonstrating the long-term neuroprotective effect of cilostazol in the spinal cord. Vehicle-treated and cilostazol-treated animals had little difference in intramedullary vacuolation and venous dilation. This finding suggests that cilostazol may be acting mainly with mechanisms other than improvement in circulatory disturbance under the mechanical compression sustained for 25 weeks. Some investigators, observing limited improvement of blood flow in cerebral hypoperfusion models with this drug, assumed that the protective effect of cilostazol might be independent of changes in blood flow.

Previous histopathological studies on cord compression in the hyperostotic mouse (twy/twy) showed apoptosis of oligodendrocytes in the gray and white matter at the most compressed site. These findings were similar to those in human autopsy studies of ossification of the posterior longitudinal ligament. Loss of oligodendrocytes caused by apoptosis is postulated to contribute to long-tract degeneration of the spinal cord. Spinal cord cell apoptosis may participate in the pathogenesis of cervical spondylotic myelopathy. Our study showed that cilostazol treatment suppressed the number of TUNEL-positive cells. The antiapoptotic effect of the drug may contribute to neuroprotection in the chronic compressed cord.

In clinical use, long-term administration of cilostazol is generally well tolerated. However, untoward side effects, such as headache, palpitation, and diarrhea, may limit its use, although these effects are usually mild in intensity. The incidences of these side effects are considered to be not greater than 10%. The inhibition of platelet aggregation may cause a bleeding tendency. The CSPS I and II demonstrated that the continuous administration of cilostazol is safe without increments in the incidence of serious hemorrhagic and cardiac events. In our study, none of the cilostazol-treated rats had diarrhea and none died. During the entire study period, no statistically significant difference in body weight or voluntary movement activity was observed between the 4 groups of animals. The gradual decrement in voluntary activity without significant intergroup differences indicated the course of habituation or maturation of the animals without the adverse effect of the drug. These findings show that the long-term oral administration of cilostazol at a dose of 30 mg/kg, which corresponds to a usual clinical dose, is safe in the animals.

Our study has several limitations. In this model, chronic spinal cord compression was induced by the implantation of a gradually expanding, water-absorbing polyurethane sheet. This polymer sheet induces no obvious tissue reactions. Furthermore, unlike other animal models, this model requires no stepwise manipulations to induce and cause progression of the pathology. Simplicity and homogeneity

Fig. 6. Bar graph shows the number of anterior horn motor neurons in the C5–6 spinal cord segment stereologically counted in a 3000-µm length. Neuronal loss was prevented significantly in Group D. Asterisks denote significant differences between groups (p < 0.05).

Fig. 7. Photomicrographs of TdT-mediated dUTP-biotin in situ TUNEL staining in Group C rats. Sections in the anterior horn (left) and anterior column (right) are shown. Arrowheads indicate TUNEL-positive cells. Bar = 100 µm.

Fig. 8. Quantification of TUNEL-positive cells in the C5–6 spinal cord. Significant differences in the number of TUNEL-positive cells in the gray and white matter were observed between Groups C and D. The x axis represents the anatomical site. AC = anterior column; AH = anterior horn; LC = lateral column; PC = posterior column; PH = posterior horn.
are the advantages of this model. To cancel out the effect of polymer expansion in the sham-operated control group, it would be better to remove the polyurethane sheet after its complete expansion. However, removing the expanded epidural sheet through the interlaminar space is impossible because the thickness of the sheet is larger than the interlaminar space. To safely remove the sheet, we must perform a C5–6 laminectomy (partially or entirely), which would add a new perturbing factor to the comparison. Therefore, we allocated the sham-operated group to receive the polymer sheet, which was immediately removed. Our previous study using the same protocol for the sham-operated group demonstrated that the alterations in motor function in the acute phase following implantation of the polymer sheet were comparable between the sham-operated and the compression groups.20 Voluntary exercise activity decreased for 1 week after the surgery and recovered in 2 weeks in both the sham-operated and compression groups. The decrement and the course were identical in both groups. Forced locomotion capability was not altered in the acute phase in either group. These results indicated that the acute effects of surgery, such as general anesthesia and exposure through the neck musculature, were more significant than the inflation of the polymer sheet, which was to occur only in the compression groups following surgery. A second consideration is that our results have limited clinical applicability. The present study was designed as a pilot study to investigate the potential efficacy of cilostazol in preventing the development of myelopathy in the cervical cord under chronic compression. To evaluate the potential efficacy of the agent as a treatment for full-blown myelopathy, further experiments must be conducted, with initiation of the drug after the onset of symptoms. In clinical practice, many patients are referred because of cervical cord compression on imaging studies and mild sensorimotor symptoms or nonfocalizing complaints, such as neck pain and shoulder strain. Many patients, despite impingement of the spinal cord, stay asymptomatic for months or years until they start to suffer from sensorimotor deficits caused by myelopathy. Most patients with cervical spondylotic myelopathy are elderly and carry higher risks for surgical decompression due to systemic medical conditions. Based on the cumulative experiences using cilostazol, it is known that chronic treatment at the dose administered in the present study (30 mg/kg/day) is safe. The drug may be useful in a condition in which impingement of the cord is evident but myelopathy is not fully symptomatic. Patients may benefit from the potential effect of prophylactic protocols.

Conclusions

We showed that early intervention with cilostazol at a clinical dose prevents the development of symptomatic myelopathy in a chronic cord compression model in animals. Progressive neuronal loss and deterioration in motor function were effectively prevented. The results indicated that continuous oral administration of cilostazol exerts a neuroprotective effect and suggests its potential therapeutic usefulness in the treatment of cervical spondylotic myelopathy.

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