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Effects of Cilostazol on Oxidative Stress, Systemic Cytokine Release, and Spinal Cord Injury in a Rat Model of Transient Aortic Occlusion

Tunay Kurtoglu,[1] Harun Basoglu,[2] Erdem Ali Ozkisacik,[1] Nesibe Kahraman Cetin,[3] Canten Tataroglu,[3] Cigdem Yenisey,[4] and Berent Discigil,[1] Aydin, Turkey

Background: Cilostazol is a phosphodiesterase inhibitor that has anti-inflammatory potential in addition to vasodilator and antiplatelet effects. The aim of this study was to determine the influence of cilostazol on biochemical markers of oxidative damage, proinflammatory cytokine release, and spinal cord injury after transient aortic occlusion in rats.

Methods: Animals were randomized into 3 groups. Sham group rats were subjected to laparotomy without aortic occlusion. Control group rats were pretreated with intraperitoneal dimethyl sulfoxide, and cilostazol group rats received intraperitoneal cilostazol (20 mg/kg/day) for 3 days before the induction of ischemia. Ischemia was induced by clamping of the infrarenal aorta, and 48 hours after reperfusion, Tarlov grades were assessed and spinal cord conduction velocities (SCCVs) were measured using epidural electrical stimulation. Erythrocyte superoxide dismutase (SOD) and catalase activities and plasma malondialdehyde, serum tumor necrosis factor−α, interleukin-1β, and interleukin-6 levels were analyzed. Spinal cord histopathology was examined to determine neuronal damage and tissue inflammation.

Results: Aortic occlusion caused significant increases in SOD, catalase activities, and malondialdehyde and cytokine levels accompanied by spinal cord injury. Cilostazol significantly reduced malondialdehyde levels but did not significantly alter the activations of antioxidant enzymes, levels of proinflammatory cytokines, or histologic severity of inflammation. The differences regarding the results of Tarlov grading, SCCVs, and neuronal viability between the ischemic and cilostazol pretreated groups were statistically nonsignificant.

Conclusion: The present experimental study indicated that cilostazol pretreatment used in this study before aortic occlusion decreased lipid peroxidation, which may be related to the reduction of reactive oxygen species. Cilostazol did not significantly suppress systemic cytokine release and prevent spinal cord inflammation and injury; however, it did show some benefit. Additional investigations might be needed to determine the critical dose of cilostazol for clarifying the protective role of this drug in spinal cord ischemia/reperfusion injury.

INTRODUCTION

Aortic cross-clamping−induced spinal cord ischemia/reperfusion (I/R) injury is a serious and unpredictable complication of thoracic and thoracoabdominal aneurysm repair. The risk of acute or delayed paraplegia as a consequence of spinal cord ischemia is reported to range from 3−18%.[1]

[1]*Department of Cardiovascular Surgery, Faculty of Medicine, Adnan Menderes University, Aydin, Turkey.*

[2]*Department of Biophysics, Institute of Health Sciences, Adnan Menderes University, Aydin, Turkey.*

[3]*Department of Pathology, Faculty of Medicine, Adnan Menderes University, Aydin, Turkey.*

[4]*Department of Biochemistry, Faculty of Medicine, Adnan Menderes University, Aydin, Turkey.*

Correspondence to: Tunay Kurtoglu, MD, Department of Cardiovascular Surgery, Faculty of Medicine, Adnan Menderes University, Aydin, Turkey; E-mail: drtunaykurtoglu@yahoo.com

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Advancements in surgical techniques and pharmacologic interventions have resulted in remarkable improvements that reduce spinal cord I/R injury during aortic surgery; however, there is currently no single therapy that eliminates neurologic dysfunction caused by aortic occlusion.[2]

Ischemia that is induced by aortic occlusion causes a drastic increase in the formation of reactive oxygen species (ROS), which in turn activates the accumulation of neutrophils and the production of various immune mediators.[3] Clamping of the aorta during surgical repair of thoracoabdominal aneurysms has been observed to trigger an inflammatory response that is conducted by systemic cytokine release.[4] In addition, increased concentrations of inflammatory cytokines have been found to be correlated with the frequency and magnitude of postoperative organ dysfunction after aortic aneurysm repair.[5]

Recent studies also concluded that the increase in serum levels of proinflammatory cytokines after prolonged aortic occlusion is accompanied by prominent motor dysfunction and evidence of histopathologic damage to the spinal cord.[6,7] Tumor necrosis factor-α (TNF-α) and interleukins-1β and -6 (IL-1β and IL-6) are key proinflammatory cytokines that play critical roles in postischemic inflammatory injury to the spinal cord.[8] These cytokines promote production of other cytokines and lead to leukocyte—endothelial cell interaction via the expression of endothelial leukocyte adhesion factor-1, which eventually results in endothelial cell damage and spinal cord injury.[9,10]

Cilostazol is a quinolinone derivative that potently inhibits phosphodiesterase type 3 and thereby increases the intracellular level of cyclic adenosine monophosphate.[11] It is extensively used for the relief of ischemic symptoms in peripheral vascular disease because it induces relaxation in vascular smooth muscle cells and inhibits platelet aggregation.[12,13] Nevertheless, a growing amount of information implicates that cilostazol possesses an anti-inflammatory potential in addition to the vasodilator and antiplatelet effects. Cilostazol has been shown to decrease the production of proinflammatory mediators and suppress inflammatory responses in various disorders.[14—16] It has been suggested that the reduction of ROS and subsequent inhibition of cytokine generation play an important role in the anti-inflammatory activity of cilostazol.[17,18]

In a recent experimental study, cilostazol treatment has been reported to cause a reduction of oxidative stress and histologic severity of injury in spinal cord tissue.[19] However, to our knowledge, the effects of cilostazol on the systemic inflammatory response induced by aortic occlusion has not been investigated. We suggest that cilostazol may suppress the systemic inflammatory response via a reduction of oxidative damage and therefore attenuate spinal cord injury in aortic occlusion—induced ischemia.

The aim of this experimental study was to determine the effects of cilostazol pretreatment on biochemical markers of oxidative stress and levels of proinflammatory cytokines in a rat model of transient aortic clamping. In order to assess the effects of cilostazol on I/R-induced spinal cord injury, hind limb motor functions were graded, spinal cord conduction velocity (SCCV) was measured, and spinal cord histology was examined.

METHODS

Animals and Groups

The experimental design and protocol were approved by the Animal Care Committee of Adnan Menderes University, and the animals were treated in accordance with National Institute of Health's Guide for the Care and Use of Laboratory Animals. Adult male Sprague—Dawley rats weighing 290—320 g were purchased from Kobay A.S. Experimental Animals Laboratory (Ankara, Turkey) and were fed a standard laboratory diet and water ad libitum and housed at controlled room temperature (24.5—25°C) and in a 12-hour dark/light cycle before and during the study.

Cilostazol (C 0737) and dimethylsulfoxide (DMSO; D 5879) were purchased from Sigma-Aldrich (St. Louis, MO). Cilostazol was first dissolved in DMSO and then diluted with physiologic saline just before the injection.

Animals were randomized into 3 experimental groups: (1) sham group rats, subjected to laparotomy without aortic occlusion; (2) control group rats, pretreated with intraperitoneal DMSO before the induction of aortic occlusion; and (3) cilostazol group rats, treated with intraperitoneal cilostazol for 3 days before aortic occlusion, with a total daily dose of 20 mg/kg in 2 equally divided doses. We preferred to include 8 animals in each group based on previous experimental rodent studies.[19,20]

Experimental Model of Spinal Cord Ischemia

Rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg) and were maintained on

an additional dose of ketamine as required. A rectal temperature probe was used to monitor body temperature, which was kept at 35.5−37.5°C with a heat lamp. The tail artery was cannulated with a 24-gauge catheter for the monitoring of distal arterial pressure and obtaining of samples for blood gas analysis during the procedure. The tail vein was cannulated for intravenous fluid replacement (0.9% NaCl solution) and a single dose of intravenous cephazolin sodium (10 mg/kg) was administered immediately before the surgery for infection prophylaxis.

Spinal cord ischemia was induced by transient occlusion of the abdominal aorta according to a previously described method.[21] In brief, the rats were placed in the prone position and a midline laparotomy was performed. Sham group rats were subjected to laparotomy without aortic occlusion. In the control and cilostazol groups, the segment of aorta between the left renal artery and aortic bifurcation was exposed, and intravenous heparin (150 U/kg) was administered before aortic clamping. The abdominal aorta was then occluded with vascular clamps (Vascu-Statt II- Scanlan, St. Paul, MN) for 45 min, placing 1 clamp just distal to renal artery and the other proximal to the aortic bifurcation. After an ischemic period of 45 min, the clamps and catheters were removed and the abdomen was closed by using silk sutures in a double-layer manner.

The animals were then returned to their cages for recovery with free access to food and water. Acetaminophen (0.25 mg/mL) was added into the drinking water for postoperative analgesia. The Crede maneuver was used to empty the urinary bladder twice a day.

Neurologic Assessment

The hind limb motor functions of the rats were examined and graded by an independent observer 48 hours after reperfusion when the animals were fully conscious. The neurologic assessment was performed according to the modified Tarlov scoring system: (0) no voluntary movement (complete paraplegia); (1) perceptible movement at the joint; (2) good joint mobility but unable to stand; (3) ability to stand but unable to walk; (4) weak walking; (5) complete recovery.[6,22]

Analysis of Spinal Cord Conduction Velocity

The rats were reanesthetized after neurologic assessment to determine SCCV. The measurement of SCCV was performed according to the previously described method by Basoglu et al.[23] Briefly, a longitudinal midline dorsal incision was made and the skin overlying the lumbosacral vertebrae was removed as a flap. Monopolar needle electrodes (EL400; Biopac Systems, Goleta, CA) were sequentially inserted at L1−2 and L2−3 dorsal intervertebral spaces for proximal stimulation and L5−6 and L6−S1 dorsal intervertebral spaces for distal stimulation. The skin surface of the left gastrocnemius muscle was shaved and cleaned with 70% ethanol and 4-mm diameter shielded silver/silver chrloride surface recording electrodes (EL254S; Biopac Systems) were plastered to the skin surface of the muscle to obtain compound muscle action potentials (CMAPs). All stimulations and recordings were processed using the MP100 data acquisition system (Biopac Systems).

A supramaximal electrical stimulus (intensity: 10 volts, current: 10 mA DC, duration: 0.1 ms) was applied at first to proximal stimulation electrodes and then to distal stimulation electrodes. SCCV was calculated using the formula SCCV = $\Delta x/\Delta t$, where Δx is the distance between the midpoint of proximal stimulation electrodes and the midpoint of distal stimulation electrodes and Δt is the latency difference of CMAPs that were recorded by distal and proximal stimulus. Δx was measured using a 120-mm Vernier caliper. Latency of a CMAP is determined as the time interval between the stimulus artifact and the first deflection from the baseline of electromyography signals.

Biochemical Analysis

Blood samples for biochemical analysis were obtained after the measurement of SCCV by intracardiac puncture and animals were killed by exsanguination.

Malondialdehyde (MDA) levels were measured according to the method described by Okahawa et al.[24] The reaction mixture contained solutions of 8.1% sodium dodecyl sulfate (SDS), 20% acetic acid, 0.67% thiobarbituric acid, and plasma sample. MDA bis (dimethyl acetal) (1,1,3,3-tetramethoxypropane, 10-838-3; Sigma-Aldrich) was used as external standard. The absorbance for each sample was measured spectrophotometrically (Shimadzu UV-160; Shimadzu, Tokyo, Japan) at 532 nm and the result was expressed as nanomole per milliliter.

Catalase (CAT) activity was measured by the method described by Aebi.[25] The reaction mixture contained 50 mM phosphate buffer (pH: 7.0), 10 mM H_2O_2, and eryhtrocyte lysate. The change in absorbance was followed for 30 sec at 240 nm at

15-sec intervals, and the activity of CAT was expressed as units per gram of hemoglobin (U/g Hb).

Measurements of copper/zinc−SOD activity in erythrocyte lysates were made according to the method used by Sun et al.[26] SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with nitroblue tetrazolium (NTB) to form formazan dye. The production of formazan was determined at 560 nm using a Shimadzu spectrophotometer (Tokyo, Japan). The percent inhibition is calculated as below:

$$\%\ \text{inhibition} = \left[\left(A_{\text{blank}} - A_{\text{sample}}\right)/A_{\text{blank}}\right] \times 100$$

Copper/zinc−SOD activity was calculated by comparison with the standard curve. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate.

The serum concentrations of inflammatory mediators were measured by performing enzyme-linked immunosorbent assay (ELISA) analyses. IL-1β, IL-6, and TNF-α from each sample were quantified using ELISA kits (Bender MedSystems Gmbh, Vienna, Austria) designed for rats. Results were calculated using a Bioelisa Reader Elx800 (BioTek Instruments Inc., Winooski, VT) using standard curves and expressed as pictograms per milliliter (pg/mL) of serum.

Histopathologic Evaluation

Spinal cord specimens were immediately harvested and samples were fixed in 10% formalin and embedded in paraffin blocks for sectioning. Four serial transverse sections (4-μm thickness) were prepared from L4−6 spinal cord segments, stained with hematoxylin−eosin stain, and examined under light microscopy (Olympus BX50; Olympus Corp., Tokyo, Japan). All sections were evaluated by a pathologist who was unaware of the experimental groups to determine the ratio of viable neurons in the gray matter and grade the severity of inflammation. The gray matter neurons that displayed cellular swelling or eosinophilic cytoplasm, loss of nucleus, and Nissl bodies were considered as injured. Cells that had a prominent nucleus with fine chromatins and contained cytoplasmic Nissl bodies were identified as viable cells. The viability index was calculated as the ratio among the number of normal motor neurons and the number of all motor neurons in the entire spinal cord section for each animal (viability index = number of viable cells/total number of neurons).[27] The severity of inflammation was graded for each section according to the previously described method as follows: score 0, very high infiltration of inflammatory cells with very high vascularization with hyperemia; score 1, high infiltration of inflammatory cells with high vascularization with hyperemia; score 2, moderate infiltration of inflammatory cells with moderate vascularization with or without hyperemia; score 3, low infiltration of inflammatory cells with moderate vascularization with or without hyperemia; and score 4, no infiltration of inflammatory cells with normal vascularization without hyperemia.[28] The mean value of the scores was used for analysis.

Statistical Analysis

All data were presented as the mean ± SEM and were calculated using Graphpad Prism software (version 5.0; GraphPad Software, Inc., San Diego, CA). The results among groups were compared by using the Tukey's multiple comparison test, 1-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

RESULTS

All animals survived throughout the study. Heart rate, arterial blood gases and pH, blood pressure, and body temperature were similar and stable in each group during the procedure.

Neurologic Outcome

All animals in the sham group had normal neurologic functions. On the other hand, rats in the control group had a significant decline in Tarlov score at 48 hours postoperatively ($P < 0.05$). Tarlov scores in the cilostazol group were higher compared to the control group, but the difference was statistically nonsignificant ($P > 0.05$; Fig. 1).

The mean of calculated SCCVs for all groups are shown in Figure 2. SCCV was significantly decreased in the control and cilostazol groups compared to the sham group ($P < 0.05$). There was no statistically significant difference of mean SCCVs between the cilostazol and control groups.

Histopathologic Outcome

Examination of the spinal cord samples in the control group revealed a significant reduction of viable neurons ($P < 0.05$). The histologic score of inflammation in spinal cord sections were higher in the sham group compared to the control group ($P < 0.05$), which reflects a higher degree of inflammation in

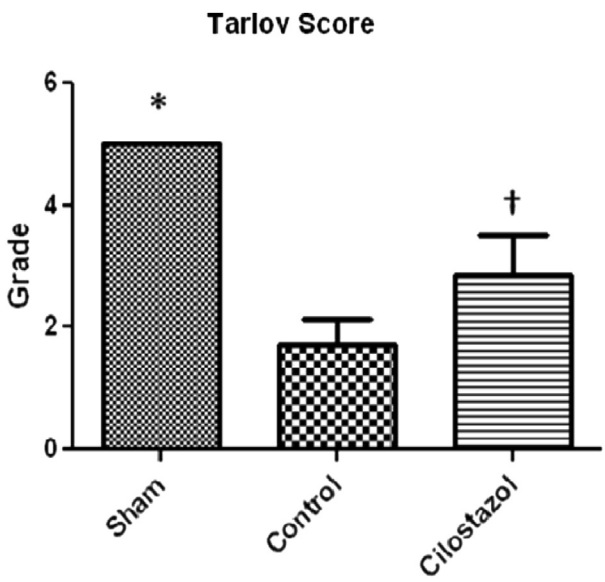

Fig. 1. Tarlov scores of groups at 48 hrs postoperatively. $*P < 0.05$ compared to control and cilostazol groups. $\dagger P > 0.05$ compared to control group.

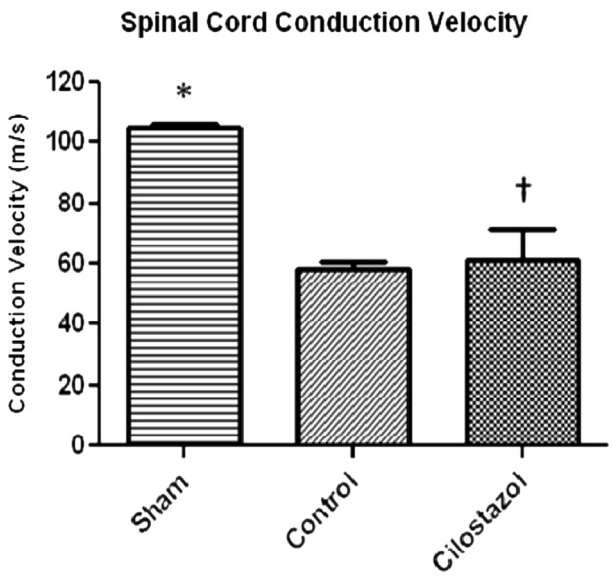

Fig. 2. Spinal cord conduction velocities of groups. $*P < 0.05$ as compared to control and cilostazol groups. $\dagger P > 0.05$ compared to control group.

the control group (Fig. 3). Viability indices and inflammation scores in the cilostazol group were higher than in the control group; however, the differences were not statistically significant (Table I).

Biochemical Results

The mean concentrations of plasma MDA levels and erythrocyte CAT and SOD activities for all groups are shown in Table II. In the control group, the MDA level was significantly higher than in the other groups ($P < 0.05$). Erythrocyte antioxidant enzyme activities (CAT and SOD) were lower in the sham group compared to the control group ($P < 0.05$). The cilostazol group had significantly higher CAT activity than the sham group ($P < 0.05$), whereas there was no significant difference between these 2 groups regarding SOD activity ($P > 0.05$).

The serum levels of IL-1β, TNF-α, and IL-6 are consecutively shown (Figs. 4–6). The levels of these cytokines were found to be significantly increased in the control group compared to the sham group ($P < 0.05$). In the cilostazol group, the levels of cytokines were lower than in the control group, but the differences were not statistically significant ($P > 0.05$).

DISCUSSION

In the present study, we investigated the effects of cilostazol administration on systemic oxidative stress, proinflammatory cytokine release, and spinal cord injury induced by transient aortic occlusion in rats. I/R caused prominent oxidative stress and an increase in proinflammatory cytokine levels. These changes were accompanied by spinal cord injury that was evident with a significant decline in SCCV, Tarlov grade, and neuronal viability in ischemic animals. In addition, I/R resulted in significant inflammation of the spinal cord as shown by histologic examination. Cilostazol administration resulted in a significant reduction of MDA, which is an indicator of lipid peroxidation, but did not significantly change the activities of the antioxidant enzymes SOD and CAT. Serum levels of proinflammatory cytokines and the degree of spinal cord inflammation were not significantly changed in the cilostazol group compared to the ischemic group. Although the differences were not statistically significant, SCCV, Tarlov grade, and the viability index were higher in the cilostazol group compared to the ischemic animals.

The decrease of blood supply to an organ causes a series of cellular and metabolic alterations known as ischemia. The restoration of blood flow to tissue after an ischemic period can result in additional damage and organ dysfunction—so-called reperfusion injury. I/R injury is primarily mediated by ROS, which can damage cell structures and cause lipid peroxidation chain reactions that eventually lead to formation of secondary end products, such as MDA, other aldehydes, and conjugated dienes.[29] SOD and CAT are antioxidant enzymes that block the harmful effects of ROS and provide endogenous

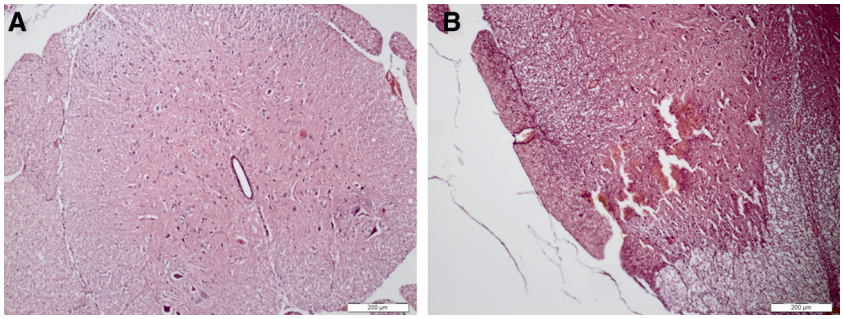

Fig. 3. Representative histologic samples from sham (**A**) and control (**B**) groups. (**A**) Normal spinal cord section. (**B**) Intraparenchymal hemorrhage with infiltration of inflammatory cells.

Table I. Histologic scores of inflammation and viability indices in spinal cord tissues

Group	Viability index (%)	Inflammation score
Sham	92.2 ± 1.4*	3.9 ± 0.1*
Control	58.0 ± 0.7	2.6 ± 0.2
Cilostazol	67.0 ± 6.6†	3.0 ± 0.3†

Data are presented as mean ± standard error of the mean.
*$P < 0.05$ compared with control group.
†$P > 0.05$ compared with control group.

Table II. Concentrations of plasma malondialdehyde levels and erythrocyte catalase and superoxide dismutase activities

Group	MDA (nmol/ml)	CAT (U/g Hb)	SOD (U/g Hb)
Sham	5.2 ± 0.6*	152.7 ± 19.1*	103.5 ± 2.7†
Control	15.1 ± 1.2	921.7 ± 114.7	138.5 ± 14.7
Cilostazol	9.8 ± 1.2†	649.0 ± 166.3	132.9 ± 7.0

CAT, catalase; MDA, malondialdehyde; SOD, superoxide dismutase.
Data are presented as mean ± standard error of the mean.
*$P < 0.05$ compared with control and cilostazol groups.
†$P < 0.05$ compared with control group.

protection against I/R injury.[30,31] SOD reduces superoxide—the primary ROS produced during I/R—to hydrogen peroxide, and then catalase enhances the degradation of hydrogen peroxide to oxygen and water.[32] Oxidative stress can cause the cells to augment such defensive enzymes in an attempt to counteract the oxidant effects and restore the redox balance.[29,33] Therefore, determination of MDA levels and the activities of antioxidant enzymes will indirectly give an idea about the amount of ROS after I/R. Several studies have shown that activities of SOD and CAT increase subsequent to an ischemic insult during the reperfusion period.[34,35] In a previous study, levels of MDA, SOD, and CAT were reported to be significantly increased in spinal cord tissue after aortic occlusion.[36] We also observed significantly higher concentrations of plasma MDA and erythrocyte SOD and CAT activities in the control group compared to the sham group, which suggests a relation with increased lipid peroxidation and activation of defensive antioxidant enzymes caused by I/R. In addition, the activities of SOD and CAT correlated well with MDA levels and the severity of spinal cord injury in all groups.

Spinal cord I/R injury involves the interaction of integrated pathophysiologic mechanisms. The generation and release of inflammatory mediators associated with increased ROS formation play an essential role in the pathogenesis of spinal cord injury.[37,38] Aside from causing direct cellular toxicity, ROS can trigger the accumulation of neutrophils in ischemic tissue and stimulate the monocytes to produce inflammatory mediators, such as IL-1β and TNF-α.[37,39] These cytokines are released into the systemic circulation upon reperfusion and act on endothelial cells to promote the production of other types of cytokines, including IL-6, which causes respiratory burst of neutrophils and enhances the release of ROS.[36,40]

It is suggested that a significant portion of ischemic spinal cord injury occurs during the reperfusion period through the activation of inflammatory cascades.[28,41] Various experimental studies have shown that systemic cytokine release in response to aortic occlusion is closely linked with the severity of spinal cord injury. Hasturk et al.[7] concluded that serum proinflammatory cytokines (i.e., TNF-α, IL-1β, and IL-6) significantly increase after spinal cord I/R injury accompanied by tissue damage. The increased serum levels of cytokines induced by infrarenal aortic occlusion were observed to be associated with a deterioration of motor functions and signs of histologic damage to spinal cord.[6] In addition, systemic levels of IL-6

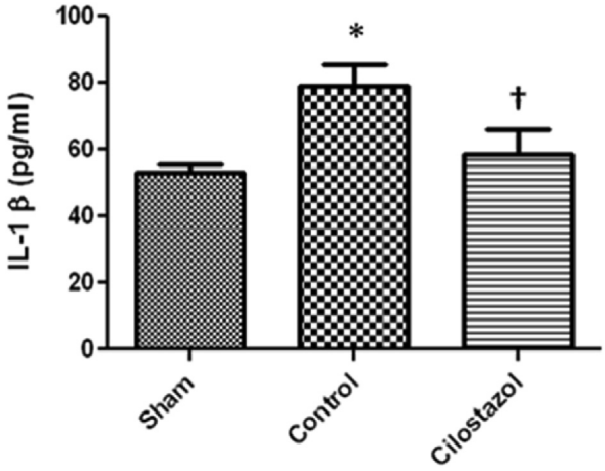

Fig. 4. Serum interleukin-1β levels in groups. *$P < 0.05$ compared to sham group. †$P > 0.05$ compared to control group.

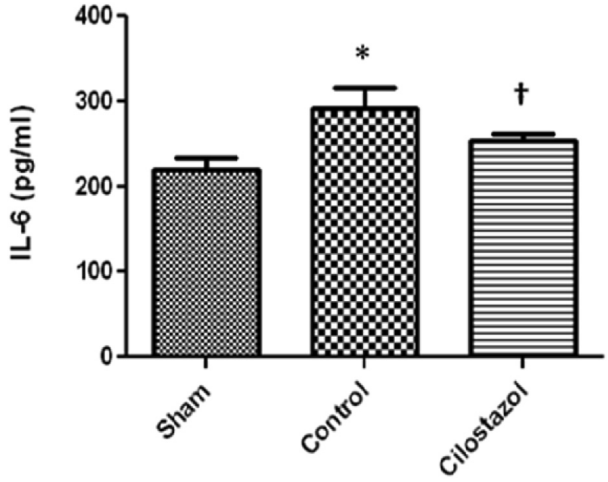

Fig. 6. Serum interleukin-6 levels in groups. *$P < 0.05$ compared to sham group. †$P > 0.05$ compared to control group.

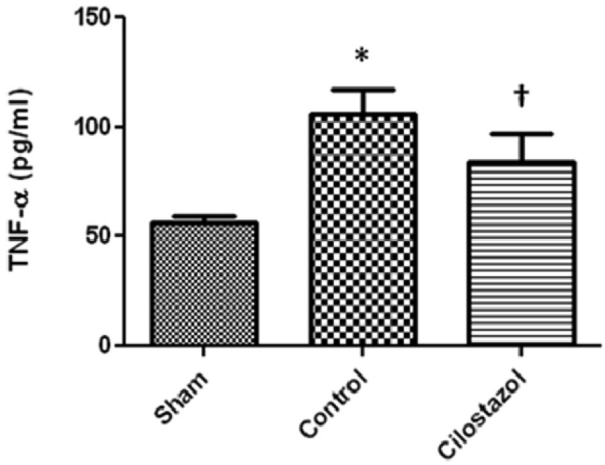

Fig. 5. Serum tumor necrosis factor−β levels in groups. *$P < 0.05$ compared to sham group. †$P > 0.05$ compared to control group.

and TNF-α were found to be significantly elevated at the end of reperfusion period in comparison with the levels before the aortic occlusion in a porcine model of spinal cord I/R injury.[42] Similar to these reports, we observed significantly increased levels of serum TNF-α, IL-1β, and IL-6 and a higher degree of inflammation in spinal cord tissue in ischemic animals compared to sham animals. We also observed that elevated levels of these cytokines were associated with more severe spinal cord injury, as was evident by lower Tarlov grades, SCCVs, and the viability index.

Phosphodiesterase inhibitors including cilostazol have been shown to inhibit cytokine production and exert potent anti-inflammatory effects.[18,43,44] There is a wealth of evidence to suggest that emergence of anti-inflammatory activity of cilostazol is associated with the reduction and elimination of ROS. Cilostazol has been reported to decrease the remnant lipoprotein particles, induced nicotinamide adenine dinucleotide phosphate oxidase−dependent superoxide formation, and TNF-α and IL-1β production in endothelial cells.[45] Similar to these data, it has been shown that cilostazol causes a significant reduction of ROS production by human umbilical vein endothelial cells exposed to lipopolysaccharide and prevents TNF-α−induced cell death.[18] In a rat model of atherosclerosis, treatment with cilostazol has been observed to attenuate plaque formation and decrease macrophage accumulation with reduced superoxide and cytokine production.[46] Cilostazol administration has also been shown to reduce laddered DNA fragmentation in ischemic rat cerebral tissues, which is an indicator of ROS-triggered apoptosis.[47] Moreover, cilostazol has been shown to possess significant ability to scavenge hydroxyl and peroxyl radicals and suppress TNF-α production in focal cerebral ischemia.[48]

The elimination half-life of cilostazol in humans is 10.5 hrs and the recommended clinical dose is 100 mg twice a day.[49] The plasma concentration of cilostazol after a single oral administration of 10 mg/kg in rats has been shown to be similar to that of the clinical dose of a single oral administration of 100 mg in humans.[20] In a rat model of myocardial ischemia, the application of cilostazol 20 mg/kg/day for a period of 3 days has resulted in decreased infarct size.[50] In addition, the intraperitoneal administration of cilostazol at a dose of 10 mg/kg has been reported to suppress the production of proinflammatory cytokines in stress-induced gastric

mucosal injury.[51] Based on these data, we preferred to administer a 10-mg/kg intraperitoneal injection of cilostazol twice a day (20 mg/kg/daily) for 3 days before aortic occlusion.

In the present study, we did not observe a significant suppression of proinflammatory cytokines with cilostazol pretreatment. We also did not note a significant change in the activities of antioxidant enzymes SOD and CAT but plasma MDA level, which is an indicator of lipid peroxidation was found to be significantly reduced after cilostazol administration. Cilostazol treatment did not result in significant increases in the Tarlov grade and in the number of viable neurons in histologic examination of the spinal cord. Nevertheless, we observed that cilostazol pretreatment caused some benefits by reducing the levels of circulating proinflammatory cytokines and decreasing the histologic severity of inflammation in spinal cord tissue. In addition, cilostazol group rats had more favorable outcomes in terms of postischemic hind limb motor functions and neuronal viability than the ischemic animals. These findings suggest that cilostazol treatment—at least to some extent—resulted in the alleviation of oxidative stress evident with decreased lipid peroxidation, which may be related to the decrease in ROS production and/or the radical scavenger effect of cilostazol. The benefits of cilostazol in attenuation of postischemic inflammatory response and spinal cord injury induced by aortic occlusion may be the result of reduced oxidative stress; however, this effect is not prominent.

Several factors could be suggested to explain the failure of cilostazol treatment in producing the anticipated effect in the present study design. The dose of cilostazol used in this experimental study has been adjusted to mimic the commonly used clinical dosage of the drug. In a previous experimental model of spinal cord I/R injury in rats, Sahin et al.[20] used a single oral dose of cilostazol (20 mg/kg/day). Similar to the findings of our study, they reported a reduction in biochemical markers of oxidative stress in ischemic spinal cord tissue and did not observe a significant improvement in Tarlov score with cilostazol treatment. On the other hand, the use of higher doses of cilostazol that attenuate cerebral ischemia has been reported in rats.[46,52] Although we have not investigated the dose-dependent responses in this study, we cannot exclude the possibility that application of higher doses of cilostazol could have resulted in a more potent effect with significant reduction of the systemic cytokine release and apparent clinical improvement. Another possible reason that might explain the negative results may be the relatively small number of animals included in the study. It can be suggested that increasing the number of animals in each group could have yielded statistically significant differences between the outcomes of the groups.

I/R can result in serious central nervous system dysfunction, including signal transmission.[53] We have previously shown that SCCV can be calculated via epidural electrical stimulation and shows significant decline after spinal cord I/R injury in rats.[23] In this study, aortic occlusion—induced spinal cord ischemia also caused a significant decrement of SCCV, in accordance with our previous report. Sekiguchi et al.[54] found that nerve conduction velocity improves with cilostazol treatment after chronic cauda equine compression, which is suggested to be related with augmented blood flow.[54] However, cilostazol treatment did not cause a significant improvement of postischemic SCCV in our study. On the other hand, mean SCCV in the cilostazol-treated group was higher than the ischemic group, which is parallel with the Tarlov grade and the viability index and indicates a benefit of cilostazol pretreatment.

The following limitation of the present study must be taken into account when considering the results. Although we have analyzed systemic proinflammatory cytokine release, we did not determine the levels of these cytokines in spinal cord tissues, which could have provided valuable information about the inflammatory response.

CONCLUSION

The present experimental study indicated that intraperitoneal administration of cilostazol at a dose of 20 mg/kg/daily for 3 days before aortic occlusion decreases lipid peroxidation, which may be related to the reduction of ROS but does not significantly suppress systemic cytokine release and prevent spinal cord inflammation and injury. However, cilostazol pretreatment has some benefit in alleviating the inflammatory response and ischemic spinal cord injury, which are suggested to be the result of a decrease in oxidative stress. Additional investigation might be needed to determine the critical dose of cilostazol for clarifying the protective role of this drug in spinal cord I/R injury.

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