Cilostazol Attenuates Spinal Cord Ischemia-Reperfusion Injury in Rabbits

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Objective: **The aim of this study was to evaluate the pretreatment effect of cilostazol on spinal cord ischemia-reperfusion injury.**

Design: **Prospective, interventional study.**

Setting: **Research laboratory, single institution.**

Participants: **Twenty-four New Zealand white rabbits.**

Interventions: **Twenty-four rabbits were divided into 3 equal groups: group I (sham), group II (ischemia-reperfusion, control group), and group III (cilostazol, administered orally 30 mg/kg/day for 3 days before the surgery). Spinal cord ischemia was induced by clamping the aorta both below the left renal artery and above the iliac bifurcation for 30 minutes. Seventy-two hours postoperatively, the motor function of the lower limbs was evaluated in each animal according to the modified Tarlov score. Spinal cord and blood samples were taken for histopathologic and biochemical analyses at the 72nd hour of reperfusion.**

Measurements and Main Results: **All rabbits in the ischemia-reperfusion group (group II) showed severe neurologic deficits. The median (IQR) Tarlov scores postoperatively at 72 hours in groups I, II, and III were 5.0(-), 2.0(1.0), and 4.5(1.0), respectively. Administration of cilostazol resulted in a significant reduction in motor dysfunction when compared with the ischemia-reperfusion group ($p < 0.001$). In the ischemia-reperfusion group, serum and tissue glutathione peroxidase and superoxide dismutase activity were significantly less compared with the sham group (group I) ($p < 0.05$). Serum and tissue glutathione peroxidase and superoxide dismutase levels in the cilostazol-treated group (group III) were higher compared with the ischemia-reperfusion group ($p < 0.05$). In the cilostazol-treated group, serum and tissue malondialdehyde levels were lower compared with the ischemia-reperfusion group ($p < 0.05$). Histopathologic analysis found decreased neuronal injury in the cilostazol group when compared with the ischemia-reperfusion group ($p < 0.05$).**

Conclusions: **This study showed that pretreatment with cilostazol significantly ameliorated neurologic functional outcome and attenuated neuronal histopathologic injury after transient aortic occlusion in rabbits.**

KEY WORDS: cilostazol, spinal cord, ischemia-reperfusion injury, spinal cord protection, paraplegia

AFTER THORACIC OR ABDOMINAL AORTA surgery, there is a well-known risk of paraplegia due to spinal cord injury caused by ischemia-reperfusion (I/R). Paraplegia has been reported in remarkably high incidences ranging from 2.4% to 40%.[1] This destructive complication has been ascribed, at least in part, to temporary or permanent ischemia of the spinal cord caused by the decline or interruption of the blood flow during aortic cross-clamping.[2] In an attempt to prevent this complication, various methods for spinal cord protection during the ischemia-reperfusion period have been suggested involving temporary shunts or partial bypass, hypothermia, cerebrospinal fluid drainage, ischemic preconditioning, and pharmacologic interventions.[3–6] Regardless of the surgical technique or method of spinal cord protection used, no method has prevented completely the development of paraplegia. In recent years, research on pharmacologic measures reducing spinal cord I/R injury have increased because of greater ease of their administration.[7] Current pharmacological research studies involve the use of steroids, oxygen-derived free radical scavengers, vasodilators, and various drugs designed to achieve spinal cord electrical silence during the ischemia-reperfusion period.[3,4] It is important to state that none of the pharmacologic protective strategies have been shown clearly to significantly reduce the risk of spinal cord injury during surgery on the aorta.

Cilostazol has been used worldwide to treat the symptoms of lower extremity peripheral arterial disease. Cilostazol increases the intracellular level of cyclic adenosine monophosphate by inhibiting its hydrolysis by type-III phosphodiesterase.[8] The precise mechanisms by which cilostazol exerts its beneficial effects are not completely understood, but most likely are the consequences of antiplatelet, antithrombosis, and vasodilatory effects.[9–11] Although to date cilostazol has not been shown to have neuroprotective properties in human spinal cords, it has been reported that cilostazol has neuroprotective effects in ischemic cerebral injury, diabetic retinal vascular dysfunction, neuronal degeneration, and chronically compressed cervical spinal cord in several recent experimental studies.[12–16] This neuroprotective potency of the cilostazol has been considered to be associated with its anti-inflammatory, anti-oxidant and anti-apoptotic effects mediated by its free radical scavenger properties. However, it has been used as the oral and intraperitoneal agent for the prevention of ischemic spinal cord injury in 2 different experimental studies, and different results have been obtained.[17,18] Therefore, the effect of cilostazol on the spinal cord ischemia-reperfusion injury requires further investigation because of controversial results.

The aim of this study was to assess the neurologic, biochemical, and histopathologic effects of cilostazol in an ischemia-reperfusion model of spinal cord ischemia induced by cross-clamping of the infrarenal abdominal aorta.

METHODS

The study protocol was approved by Ankara Education and Research Hospital, Ethics Committee for Animal Researches, Ankara, Turkey. All experimental animals received humane care and treatment

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in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996.

Experimental Protocol

Experiments were performed on 24 New Zealand white rabbits weighing between 2.4 and 3.5 kg. They were housed in an animal room at 22ºC to 24ºC and given free access to commercial rabbit chow and tap water. The animals were followed for 10 days and were neurologically intact before the procedure. The animals were divided randomly into 3 groups with 8 animals in each group:

Group I. Sham group (n = 8); spinal cord tissue and blood samples were obtained 72 hours after only a simple laparotomy.
Group II. I/R (control) group (n = 8); spinal cord tissue and blood samples were obtained after 30 minutes of ischemia and 72 hours of reperfusion.
Group III. Cilostazol group (n = 8); the animals received cilostazol (orally via gavage 30 mg/kg/day) for 3 days before the surgical procedure. Spinal cord tissue and blood samples were obtained after 30 minutes of ischemia and 72 hours of reperfusion.

In the sham group, only laparotomy was performed without aortic cross-clamping. In the cilostazol group, 30 mg/kg/day of cilostazol (Pletal, Abdi Ibrahim Ilac Sanayi ve Ticaret A.S.) were administered orally for 3 days before the surgery. Cilostazol was dissolved in dimethyl sulfoxide (30%) immediately before use and administered within a half hour. A similar amount of dimethyl sulfoxide (30%) solution was also administered orally in the sham and I/R group.

Rabbits initially were anesthetized with intramuscular ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg), followed by a half-dose as required during the procedure. A standard amount of anesthetic agent was used in all groups. The animals were allowed to breathe room air without mechanical ventilation. Body temperature was maintained close to 38°C using a heating lamp. The ear vein and artery were cannulated before surgery. Preoperative cefazolin (10 mg/kg) was given in a single dose. After sterilization, the abdomen was entered through a median laparotomy and the abdominal aorta was exposed inferiorly to the left renal artery and down to the bifurcation. The aorta was encircled with a silk ligature distal to the renal artery and proximal to the bifurcation to facilitate secure occlusion. Each rabbit was anticoagulated with 150 IU/kg of heparin before aortic cross-clamping. The aorta was occluded for 30 minutes distal to the renal artery with a pediatric vascular clamp and proximal to the bifurcation with a similar clamp in the I/R and cilostazol groups. After releasing the aortic occlusion, the abdomen was closed in layers and the animals were allowed to recover. The surgery was performed in the same fashion in the sham group, but without aortic occlusion. Hemodynamic variables (systolic and diastolic blood pressure, and heart rate) and body temperature were monitored continuously throughout the experiment. Arterial blood gas data (pH, $PaCO_2$, PaO_2, hematocrit, glucose) were measured 5 minute before ischemia, during ischemia, and after ischemia. No differences were found among the experimental groups with respect to the mean arterial blood pressure, heart rate, arterial blood gas values, or body temperature.

All animals were euthanized at 72 hours postoperatively by a lethal cardiac injection of pentobarbital (100 mg/kg). Shortly after, the spinal cord tissue and blood samples were obtained.

Neurologic Examination

The neurologic function of the rabbits was evaluated at 24, 48, and 72 hours after spinal cord injury in each animal according to the modified Tarlov score by an independent observer who was unaware of the treatment modality of the animal. The Tarlov scale grades the animals based on their ability to move, sit, and hop. The animals were classified according to the method of modified Tarlov: 0 = indicates complete paraplegia with no movement in the hind limbs; 1 = indicates that the animal has some hind limb movement; 2 = signifies that the animal can sit with assistance; 3 = means it can sit on its own; 4 = shows the animal has a weak hop; 5 = means the animal has a normal hop.[19,20]

Biochemical Analyses

Lumbosacral spinal cords and serum were used in all rabbits from each group for biochemical analysis. After spinal cord tissues were obtained, all spinal cord tissues were washed twice with cold saline solution, placed in glass bottles, labeled, and stored in a deep freeze (−80°C) until processing. Tissues were homogenized in 4 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.4) using a glass Teflon homogenizer (Ultra Turrax IKA T18 basic, Cole-Parmer North America, Vernon Hills, IL) after cutting of the spinal cords into small pieces with scissors. The small aliquot of homogenized spinal cord tissue was used for the measurement of malondialdehyde and protein. Malondialdehyde (MDA) concentrations and protein levels were recorded at this stage. The homogenate was then centrifuged at 5000 × g for 60 minutes to remove debris. The clear upper supernatant fluid was taken, and glutathione peroxidase (GSH-Px) activities and protein concentration were measured at this stage. The supernatant solution was extracted with an equal volume of an ethanol/chloroform mixture. After centrifugation at 5000 × g for 30 minutes, the clear upper layer (the ethanol phase) was taken and used in the superoxide dismutase (SOD) activity and protein assays. The measurements were made at different steps because of the fact that some analytes may undergo subsequent oxidation after advanced preparation procedures. All preparation procedures were performed at +4°C. Blood samples obtained by cardiac puncture were drawn into serum separation tubes. The blood samples were centrifuged at 1000 × g for 10 minutes at 4°C to remove serum. Protein assays were carried out as described by Lowry et al.[21] All samples were assayed in duplicate.

Tissue and serum MDA levels were measured by a method based on the reaction with thiobarbituric acid at 100°C, as previously described by Draper and Hadley.[22] The results were given as pmol/mL in serum and pmol/mg protein in spinal cord tissues.

Total SOD activity was measured according to the nitroblue tetrazolium method described by Sun et al.[23] The results were given as U/mL for serum and U/mg protein for spinal cord protein.

GSH-Px activity was measured according to the method by Paglia and Valentine,[24] in which GSH-Px activity is coupled with the oxidation of nicotinamide adenine dinucleotide phosphate by glutathione reductase. The results were given as nmol/min/mL for serum and nmol/min/mg for spinal cord protein.

Histopathologic Examination

Coronal sections of the lumbosacral spinal cord segment were cut at a thickness of 4 μm and stained with hematoxylin and eosin for evaluation of structural changes. Neuronal injury was evaluated at 400× magnification with light microscopy by an observer who was blind for treatment groups.

The grading of the grey matter ischemic change was as follows: Grade 0: normal spinal cord; grade 1: Axonal swelling with 1-5 eosinophilic neurons in gray matter; grade 2: Axonal swelling with 5-10 eosinophilic and necrotic neurons in gray matter; grade 3: Axonal