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Activated Protein C Reduces the Ischemia/Reperfusion-Induced Spinal Cord Injury in Rats by Inhibiting Neutrophil Activation

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Objective

To examine whether activated protein C (APC) reduces spinal cord injury in rats by inhibiting neutrophil activation after the transient ischemia.

Summary Background Data

Ischemic spinal cord injury is an important pathologic mechanism leading to the paraplegia observed after surgery to repair aortic aneurysms. Activated neutrophils play a pivotal role in the development of ischemia/reperfusion-induced tissue injury. Recently, the authors have reported that APC, a physiologic anticoagulant, prevents lipopolysaccharide-induced pulmonary vascular injury by inhibiting neutrophil activation. These observations strongly suggest that APC reduces ischemia/reperfusion-induced spinal cord injury by inhibiting neutrophil activation.

Methods

In rats, spinal cord ischemia was induced by using a balloon catheter placed into the aorta. After the transient ischemia, survival and motor function were evaluated, and histologic examination of the spinal cord was performed by using both hematoxylin-and-eosin staining and 2,3,5,-triphenyltetrazolium chloride (TTC) staining 24 hours after the ischemia. Tissue levels of myeloperoxidase and cytokines, including tumor necrosis factor-α (TNF-α) and rat interleukin-8, were measured in six experimental groups: sham-operated, control, APC (100 μg/kg, intravenous), dansyl glutamyl-glycyl-arginyl chloromethyl ketone-treated activated factor X (DEGR-F.Xa), a selective inhibitor of thrombin generation (1 mg/kg, intravenous), nitrogen mustard-induced leukocytopenia, and diisopropyl fluorophosphate-treated APC (DIP-APC), active site-blocked APC (100 μg/kg, intravenous). APC, DEGR-F.Xa, and DIP-APC were administered intravenously 30 minutes before aortic occlusion. Control and leukocytopenic rats received saline instead of other drugs.

Results

Pretreatment with APC significantly reduced motor disturbances compared with those in control animals. In contrast, neither DEGR-F.Xa nor DIP-APC had any effect. Microinfarctions, evidenced by the absence of TTC staining and histologic change, were markedly reduced in animals given APC. The increases in the tissue levels of TNF-α, rat interleukin-8, and myeloperoxidase in the ischemic part of the spinal cord were significantly reduced in animals that received APC. These levels were not reduced in rats given DEGR-F.Xa or DIP-APC. Leukocytopenia produced effects similar to those of APC.

Conclusions

APC reduced the ischemia/reperfusion-induced spinal cord injury by inhibiting neutrophil activation. The therapeutic mechanisms of APC might depend on its inhibitory effect on the production of TNF-α, which is a potent activator of neutrophils. Although the anticoagulant effects of APC might not be related to its ability to inhibit TNF-α production, its serine protease activity appears to be essential in the therapeutic mechanism. APC appears to have potential as a therapeutic agent for prevention of spinal cord injury in patients undergoing aortic aneurysm repair.

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Paraplegia is a serious complication that can occur after surgical repair of thoracoabdominal aortic aneurysms.[1,2] This complication has been attributed to temporary or permanent ischemia of the spinal cord caused by interruption of the blood supply during aortic cross-clamping.[1,2] Lintott

et al[3] have reported that dissection, rupture, and prolonged clamp times are closely related to an increased incidence of paraplegia. Although various interventions, such as temporary shunts, have been performed to maintain perfusion of the spinal cord, this complication remains unpredictable and unpreventable.[3–5] Additional damage to the spinal cord may occur during the reperfusion period.[5] Recently, it has been shown that neutrophils play a central role in development of the pathologic condition of ischemia/reperfusion-induced tissue injury.[6–8] Although the role of the neutrophils in ischemia/reperfusion-induced injury of the central nervous system is not fully understood, previous studies have shown that either administration of antiintercellular adhesion molecule-1 antibody[9] or inhibition of monocyte function[10] reduced spinal cord injury after transient ischemia in animal models.

Activated protein C (APC) is an important physiologic anticoagulant that inactivates factors Va and VIIIa.[11,12] APC is generated from protein C by the action of the thrombin–thrombomodulin complex on the endothelial cells.[11] We have previously demonstrated that APC prevents pulmonary vascular injury by inhibiting neutrophil activation through inhibiting tumor necrosis factor-α (TNF-α) production in rats given lipopolysaccharide.[13,14] Thus, it is possible that APC may be effective in reducing ischemia/reperfusion-induced spinal cord injury in which activated neutrophils play an important role.

The purpose of the present study was to evaluate the efficacy of APC in the prevention of spinal cord injury after transient ischemia of the spinal cord in rats. The therapeutic mechanisms of APC in this animal model of spinal cord injury were further investigated.

METHODS

Materials

APC was derived from human thrombin-activated protein C and purified by carbon-exchange chromatography, as described previously.[15] Nitrogen mustard, myeloperoxidase, and diisopropyl fluorophosphate (DIP) were purchased from Sigma (St. Louis, MO), and dansyl glutamyl-glycyl-arginyl chloromethyl ketone (DEGR) from Carbiochem (San Diego, CA). All reagents used were of analytic grade.

Preparation of DEGR-Treated Activated Factor X

Factor X was purified from human plasma and activated with Russell's viper venom.[16] Activated factor X was inactivated by incubation with a 20-fold molar excess of DEGR for 30 minutes at 25°C, after which the mixture was subjected to extensive dialysis against a solution containing 20 mmol/L Tris-HCl (pH 7.4) and 100 mmol/L NaCl. DEGR-treated factor Xa (DEGR-F.Xa) has been shown to selectively inhibit thrombin generation by competing with intact factor Xa for prothrombinase complex formation.[17]

Preparation of DIP-Treated APC

APC was inactivated with DIP according to a previously described method.[18] Briefly, APC (1 mg/mL) was incubated with 10 mmol/L DIP in phosphate-buffered saline (pH 7.4) for 2 hours and then dialyzed extensively against the same buffer. The effectiveness of inactivation was monitored amidolytically by measuring the rate of hydrolysis of the chromogenic substrate S-2366 (Chromogenix AB, Stockholm, Sweden) at 405 nm. The amount of APC activity remaining was less than 1%.

Animal Model of Transient Ischemia of the Spinal Cord

The study protocol was approved by the Kumamoto University Animal Care and Use Committee. The care and handling of the animals were conducted in accordance with the guidelines of the National Institutes of Health.

Adult pathogen-free male Wistar rats (Nihon SLC, Hamamatsu, Japan) weighing 300 to 380 g were used in the experiments. During surgical preparation, body temperature was monitored using a rectal probe and maintained at 35.5° to 37.5°C with a thermal pad and a heat lamp. Under pentobarbital anesthesia (45 mg/kg, intraperitoneal; Abbott Laboratory, North Chicago, IL), a 2F Fogarty catheter (Baxter, Deerfield, CA) was inserted into the left femoral artery[19] and the balloon was placed at the end of the aortic arch. To induce spinal cord ischemia, the balloon was inflated with 0.05 mL distilled water for 20 minutes. The efficiency of the occlusion was evidenced by sustained loss of any detectable pulse measured by Doppler sonography in the right femoral artery. At the end of the ischemic period, the catheter was deflated and removed. After closing the wound, animals were returned to the cages and allowed to recover. This technique creates spinal cord injury in the lumbosacral segment, which causes paraplegia in a reproducible manner.[19,20]

Experimental Design

We first examined the effects of various doses of APC (10, 50, 100, and 200 μg/kg, intravenous) on the motor disturbances in rats subjected to the transient ischemia-induced spinal cord injury. To elucidate the therapeutic mechanism of APC, rats were divided into six experimental groups: sham operation; control; APC; DEGR-F.Xa, a selective inhibitor of thrombin generation; leukocytopenia; and DIP-APC, active site-blocked APC. In sham-operated animals, the balloon catheter was placed in the aorta but was not inflated. APC, DEGR-F.Xa, and DIP-APC were administered intravenously to rats 30 minutes before induction of spinal cord ischemia. Control and nitrogen mustard-induced

leukocytopenic rats received saline instead of APC or other drugs.

Evaluation of Survival and Neurologic Status

To evaluate the effect of APC on survival, animals were allowed to survive for 21 days. Animals that never recovered completely from the surgery and then died within 24 hours after reperfusion were excluded from the analyses. The motor function of rats was assessed in a masked manner using the Tarlov scale[21] and the inclined-plane test.[22] The Tarlov motor scale is as follows: 0, no voluntary movement (complete paraplegia); 1, perceptible movement at the joint; 2, good joint mobility but inability to stand; 3, ability to stand and walk; and 4, complete recovery. For the inclined-plane test, we used a smooth-surface inclined plane, and the maximum inclination of the plane at which rats could maintain themselves for 5 seconds without falling from the plane was recorded.

Staining with 2,3,5,-Triphenyltetrazolium Chloride

Staining with 2,3,5,-triphenyltetrazolium chloride (TTC) has been shown to be a reliable method for the detection and quantification of infarction in the central nervous system.[23,24] The lumbar enlargements of the spinal cord were removed from rats killed 24 hours after the transient ischemia, cut into 3-mm coronal sections, and immersed in a 2% TTC solution at 37°C for 30 minutes, followed by fixation in 10% phosphate-buffered formalin. The rostral surface of the TTC-stained section was photographed.

Histopathologic Examination of the Spinal Cord

Rats were anesthetized with pentobarbital and perfused transcardially with 10% buffered formalin for histopathologic examination 24 hours after the transient ischemia. The lumbar enlargements of the spinal cord at L1 were harvested and postfixed in the same fixative overnight. The specimens were embedded into paraffin, and transverse sections 5 μm thick were stained with hematoxylin and eosin. Samples were analyzed by a pathologist who did not know to which experimental groups the animals belonged. With hematoxylin-and-eosin staining, when significant injury was present, motor neuron cells in the ventral gray matter showed eosinophilic, structureless cytoplasm and loss of nuclear hematoxylin stainability.[24] The cells were considered viable if they demonstrated basophilic stippling. The total number of intact motor neurons in the ventral gray matter region was counted using five serial sections of each animal.

Assay of Myeloperoxidase Activity in Spinal Cord Tissue

The presence of myeloperoxidase, an enzyme specific for neutrophils, was used as an index of neutrophil accumulation in the spinal cord.[25] After the indicated period of reperfusion, spinal cords were removed, immediately plunged into liquid nitrogen for rapid freezing, and stored at −80°C for later biochemical analysis. Myeloperoxidase activity was measured at five segmental levels (cervical, upper thoracic, lower thoracic, lumbar, and sacral segments) by a modification of a previously described method.[26] The frozen sample was weighed, homogenized (1:10, w/v) in 100 mmol/L phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide (Sigma) in an ice bath and sonicated for 30 seconds. After centrifugation (2,000*g* at 4°C for 20 minutes), the supernatant was assayed for myeloperoxidase activity. The assay was started by adding 0.1 mL sample to 0.6 mL 100 mmol/L phosphate buffer (pH 6.0) containing 1.25 mg/mL o-dianisidine and 0.05% hydrogen peroxide. The change in absorbance at 460 nm after 20 minutes was measured using a spectrophotometer (DU-54; Beckman, Irvine, CA), and the myeloperoxidase activity in each sample was calculated using a standard curve prepared using purified myeloperoxidase.

Measurement of Levels of TNF-α and Rat Interleukin-8 in Spinal Cord Tissue

Levels of TNF-α and rat interleukin-8 in spinal cord tissue were determined by a modification of the method of Clark et al.[27] Briefly, the frozen sample was weighed and homogenized (1:10, w/v) in 100 mmol/L phosphate buffer (pH 7.4) containing 0.05% sodium azide in an ice bath. The homogenate was sonicated for 30 seconds and centrifuged (2,000*g* at 4°C for 20 minutes). The concentrations of TNF-α and rat interleukin-8 in the supernatant were determined using enzyme-linked immunosorbent assay kits for rat TNF-α (Biosource, Camarillo, CA) and for rat interleukin-8 (Amersham, Buckinghamshire, UK). Results are expressed as picograms of cytokine per gram of tissue.

Induction of Leukocytopenia by Nitrogen Mustard

Nitrogen mustard (1.75 mg/kg) or 0.9% saline was administered intravenously to rats 2 days before the experiments.[28] Circulating leukocyte counts on the day of the experiments were 9,375 ± 1,365 cells/μL in controls and 1,490 ± 433 cells/μL in nitrogen mustard-treated rats ($P <$ .01 vs. control). The neutrophil counts in control and nitrogen mustard-treated rats were 1,298 ± 428 cells/μL and 631 ± 161 cells/μL, respectively ($P <$ .01 vs. control). Monocyte counts at that time in control and nitrogen mustard-treated rats were 539 ± 286 cells/μL and 100 ± 57 cells/μL, respectively ($P <$ .01 vs. control).

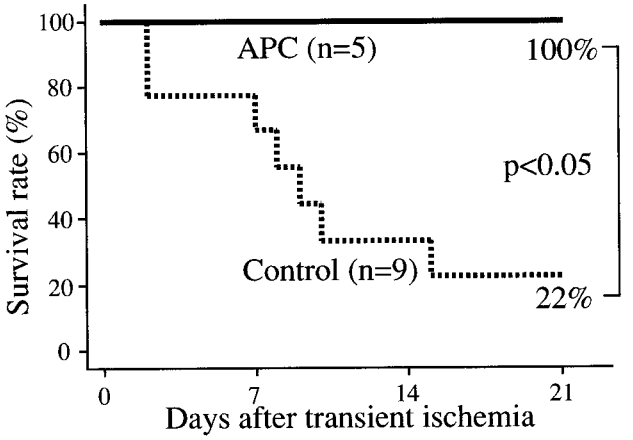

Figure 1. Survival of rats during 21 days after transient ischemia of the spinal cord.

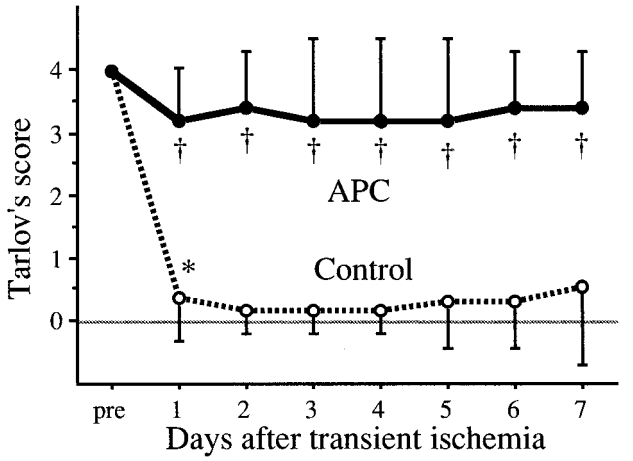

Figure 2. Changes in neurologic scores during the first 7 days after transient ischemia of the spinal cord. Neurologic grading was performed using the Tarlov scale. Open circles, control animals; closed circles, animals that received activated protein C. Data are expressed as the mean ± standard deviation from at least five animals. *$P < .01$ vs. the most recent score in the same group; †$P < .01$ vs. the other group at the same time point.

Statistical Analysis

Animal survival was assessed at 21 days after aortic occlusion, and the Fisher exact probability test was used for statistical comparison between groups. Data are expressed as mean ± standard deviation. Differences in motor scores were assessed using the nonparametric Mann-Whitney test or the Wilcoxon signed rank test. The tissue levels of myeloperoxidase, TNF-α and rat interleukin-8 were compared between two groups using the Student t test. Multiple group comparisons of mean degrees were performed using the variance followed by the Scheffe post hoc test. $P < .05$ was considered statistically significant.

RESULTS

Effect of APC on Survival and Motor Disturbances After the Transient Ischemia

Although the intravenous administration of 100 and 200 μg/kg APC significantly reduced the motor disturbances, the lower doses of APC (10 and 50 μg/kg) did not (data not shown). Thus, we examined the therapeutic effects of 100 μg/kg APC on the spinal cord injury in the present study. The overall survival rate at 21 days after aortic occlusion was significantly higher in animals given APC (100%) than in control animals (22%, $P < .05$) (Fig. 1). Although control animals showed remarkable neurologic dysfunction and did not show recovery from paralysis, the neurologic scores of animals given APC did not decrease and were significantly higher each day for 7 days than those of control animals (Fig. 2). Angles of the inclined plane were significantly higher both in animals pretreated with APC and in those with leukocytopenia than control animals 24 hours after the transient ischemia. In contrast, neither DEGR-F.Xa (1 mg/kg, intravenous) nor DIP-APC (100 μg/kg, intravenous) improved the motor disturbances (Fig. 3).

Effect of APC on the Microinfarction of the Ischemic Spinal Cord Tissue

TTC-stained normal tissue showed a well-defined border between gray and white matter, and each area was stained almost equally. Although some regions of microinfarction characterized by pale areas were observed in the tissue from control animals, the microinfarctions were remarkably attenuated in animals given APC (Fig. 4).

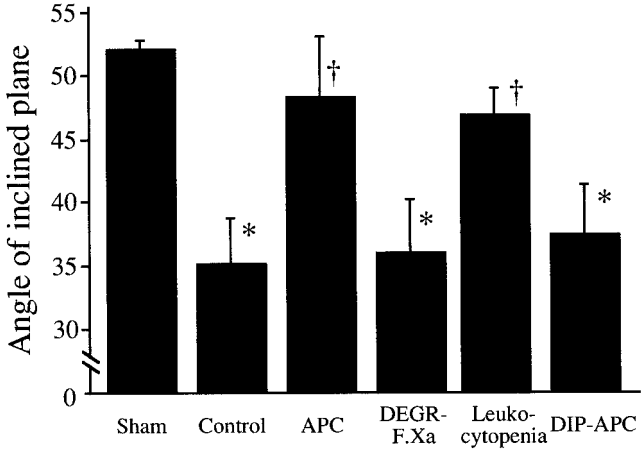

Figure 3. Effects of activated protein C (APC), dansyl glutamyl-glycyl-arginyl chloromethyl ketone-treated activated factor X (DEGR-F.Xa), leukocytopenia, and diisopropyl fluorophosphate-treated APC (DIP-APC) on motor disturbances 24 hours after transient ischemia of the spinal cord. Evaluation of motor disturbances was performed by using an inclined-plane test. Data are presented as the mean ± standard deviation from seven animals. *$P < .01$ vs. sham-operated animals; †$P < .01$ vs. control animals.

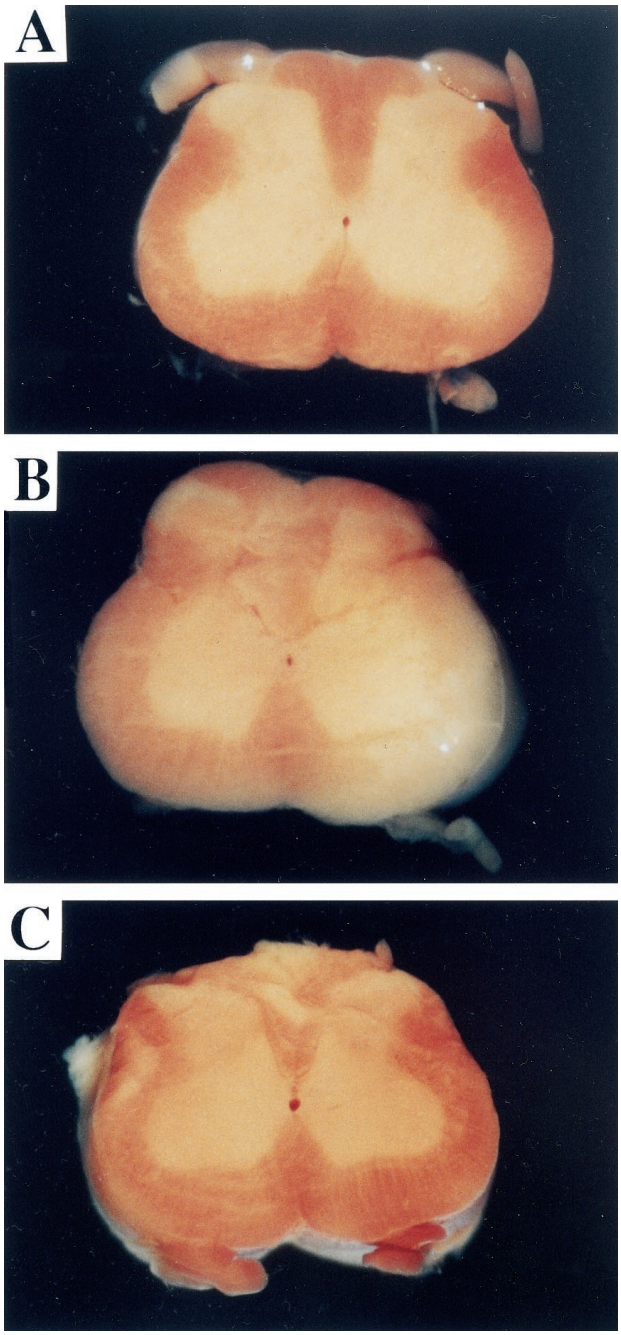

Figure 4. Photographs of 2,3,5,-triphenyltetrazolium chloride (TTC)-stained slices of spinal cord 24 hours after transient ischemia. Spinal cord tissue at the level of L1 from a sham-operated animal (A), a control animal (B), and an animal that received activated protein C (C). Typical results from each group are shown.

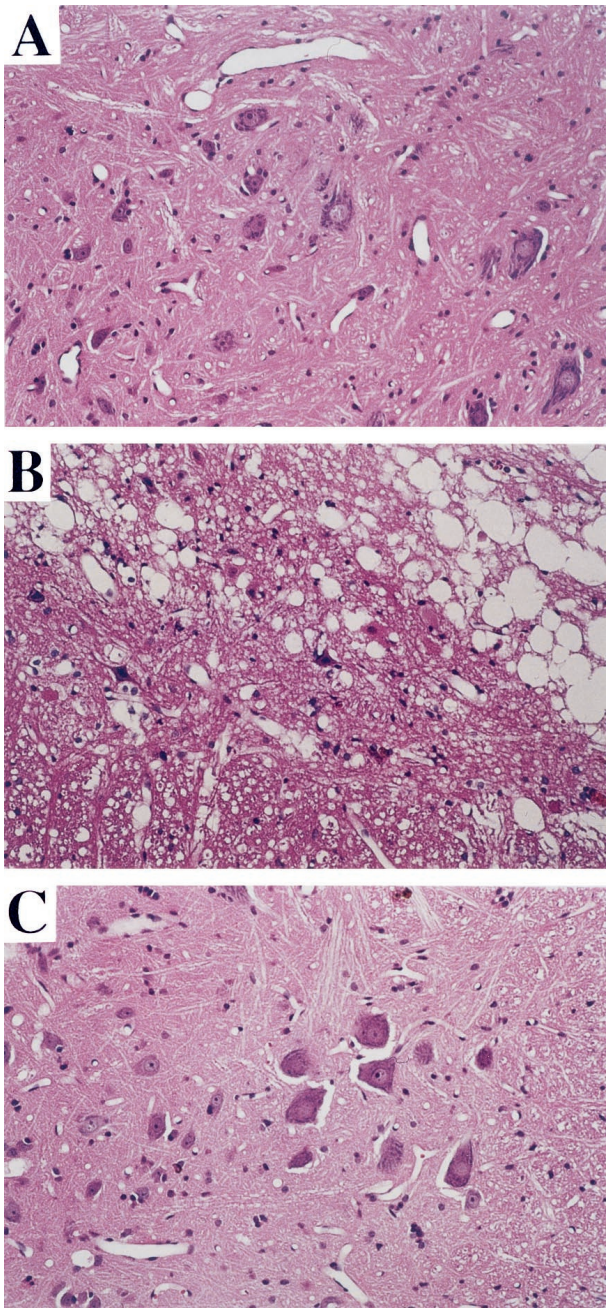

Figure 5. Photomicrographs of the lumbar enlargements of the spinal cord 24 hours after transient ischemia (×50, hematoxylin-and-eosin stain). Histology of the ventral gray matter in a sham-operated animal (A), a control animal (B), and an animal that received activated protein C (C). Typical results from each group are shown.

Histologic Observations of the Spinal Cord Tissue

No significant damage to any neurons was seen in sham-operated animals (Fig. 5). Sections from control animals showed neuronal injury characterized by cytoplasmic eosinophilia and loss of the cytoplasmic structure in the ventral gray matter. In contrast, pretreatment with APC markedly reduced these histologic changes. Although approximately 70% of motor neurons in the ventral gray matter were lost in control animals, only approximately 30% were lost in animals given APC (Fig. 6).

Changes in Tissue Levels of Myeloperoxidase in the Spinal Cord

To examine whether neutrophils accumulate in the post-ischemic part of the spinal cord, we measured the myelo-

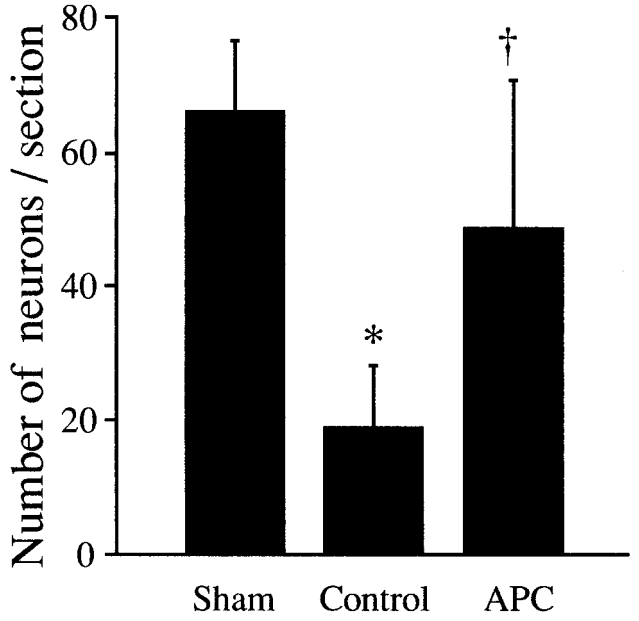

Figure 6. Effects of activated protein C on the total number of intact motor neurons in the ventral gray matter 24 hours after transient ischemia of the spinal cord. The number of intact neurons per section is presented as the mean ± standard deviation from four animals. *$P <$ .01 vs. sham-operated animals; †$P <$ .05 vs. control animals.

peroxidase activity in various spinal cord segments. The myeloperoxidase activity significantly increased in the lumbar and sacral segments but not in the cervical or thoracic segments (data not shown). Further, the myeloperoxidase activity in the lumbar and sacral segments increased with time after the aortic occlusion, peaking 24 hours after the transient ischemia (Fig. 7).

Changes in Tissue Levels of TNF-α and Rat Interleukin-8 in the Spinal Cord

The level of TNF-α was significantly increased within 1.5 hours after the transient ischemia compared with the level in sham-operated animals, peaking at 3 hours (Fig. 7). The level of rat interleukin-8 was also increased, peaking at 12 hours after the transient ischemia.

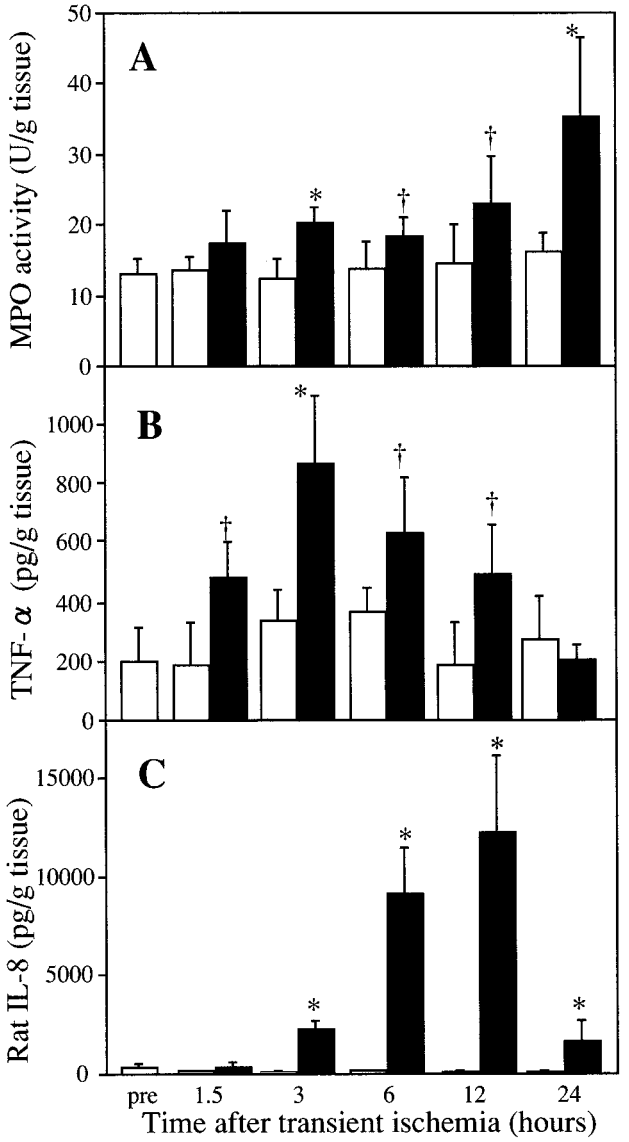

Figure 7. Time courses of spinal cord tissue levels of (A) myeloperoxidase (MPO); (B) tumor necrosis factor-alpha (TNF-α); and (C) rat interleukin-8 (TL-8). The levels were measured in the lumbar and sacral spinal cord segments before and 1.5, 3, 6, 12, and 24 hours after transient ischemia. Open bars, sham-operated animals; closed bars, control animals. Data are presented as the mean ± standard deviation from five animals. *$P <$ .01 vs. sham-operated animals; †$P <$ .05 vs. sham-operated animals.

Effects of APC on Increases in Tissue Levels of Myeloperoxidase, TNF-α, and Rat Interleukin-8

Administration of APC significantly attenuated the increases in the levels of myeloperoxidase, TNF-α, and rat interleukin-8 at the respective peak time points (Fig. 8). Although these increases at the respective peak time points were significantly inhibited in leukocytopenic animals as well, neither DEGR-F.Xa nor DIP-APC had any effect on these increases.

DISCUSSION

In the present study, the transient ischemia-induced spinal cord injury was significantly attenuated in animals that received APC (100 μg/kg) compared with control animals. Although the control animals did not recover from the motor disturbances evaluated by using the Tarlov motor scale, the motor function in animals that received APC was not decreased after the transient ischemia. APC also prevented histologic changes induced by the transient isch-

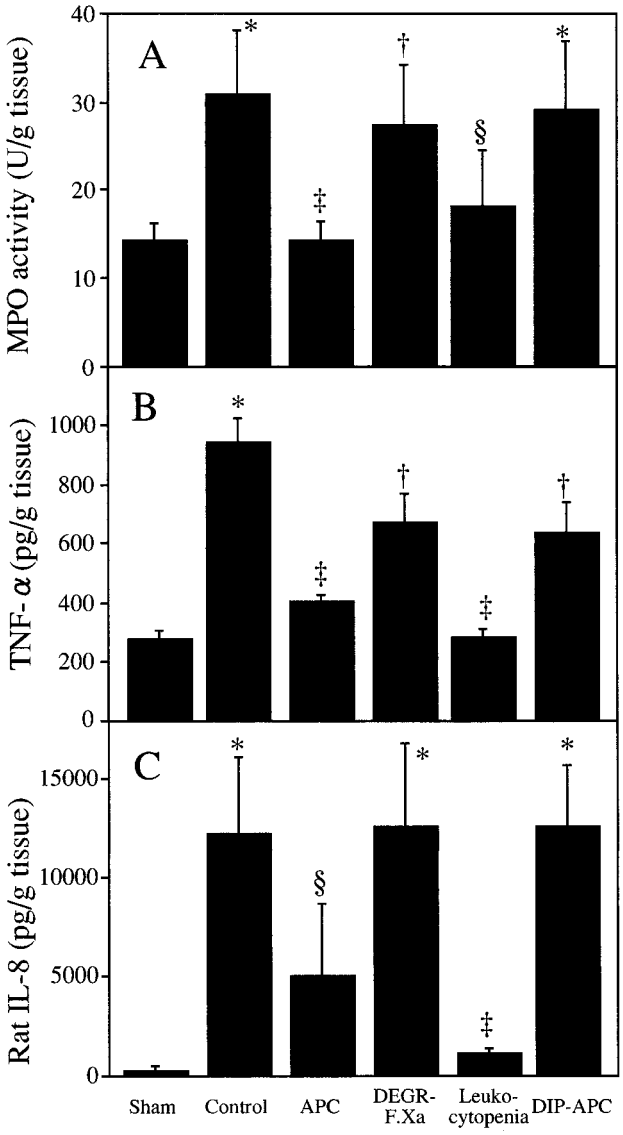

Figure 8. Effects of activated protein C (APC), dansyl glutamyl-glycyl-arginyl chloromethyl ketone-treated activated factor X (DEGR-F.Xa), leukocytopenia, and diisopropyl fluorophosphate-treated APC (DIP-APC) on increases in tissue levels of (A) myeloperoxidase (MPO), (B) tumor necrosis factor-alpha (TNF-α), and (C) rat interleukin-8 (TL-8) at 24, 3, and 12 hours, respectively, after transient ischemia. Data are presented as the mean ± standard deviation from six animals. *$P < .01$ vs. sham-operated animals; †$P < .05$ vs. sham-operated animals; ‡$P < .01$ vs. control animals; §$P < .05$ vs. control animals.

emia, such as microinfarction and loss of motor neuron cells of the ischemic spinal cord, 24 hours after the transient ischemia.

Although APC exerts its anticoagulant activity by inactivating factors Va and VIIIa,[11,12] it did not induce any adverse effects, including local or systemic hemorrhage, in animals subjected to the spinal cord injury, even at the dose of 200 μg/kg.

Although DEGR-F.Xa (1 mg/kg), a selective inhibitor of thrombin generation, inhibited lipopolysaccharide-induced coagulation abnormalities to the same extent as APC (100 μg/kg),[13,29] it failed to reduce the motor disturbances seen after transient ischemia of the spinal cord. These observations strongly suggest that APC may prevent spinal cord injury not by its anticoagulant effects, but by some other property. Because DIP-APC, an inactive derivative of APC, did not reduce the motor disturbances, the neuroprotective activity of APC might require its serine protease activity. These observations are consistent with those from our previous report showing that the attenuation of compression trauma-induced spinal cord injury by APC is independent of its anticoagulant effects but dependent on its serine protease activity.[30]

Neutrophils play a role in ischemia/reperfusion-induced tissue injury by releasing various inflammatory mediators, including neutrophil elastase and oxygen free radicals, that are capable of damaging endothelial cells.[31] Consistent with this theory, leukocytopenia significantly inhibited the neutrophil accumulation and the increases in the tissue levels of cytokines that can activate neutrophils, as well as the severity of the motor disturbances seen after transient ischemia of the spinal cord in the present study. These observations also suggested that the increases in the tissue levels of these cytokines and the neutrophil accumulation were causes rather than effects of the transient ischemia-induced spinal cord injury. Consistent with this hypothesis is a report by Giulian and Robertson[10] demonstrating that inhibition of monocyte function by chloroquine and colchicine improves neurologic outcome after temporary aortic occlusion in rabbits.

We have previously shown that neutrophil elastase plays an important role in the pathologic process leading to the compression-induced spinal cord injury by damaging endothelial cells.[32] A preliminary experiment using the present model of spinal cord injury demonstrated that ONO-5046, a neutrophil elastase inhibitor, inhibited the motor disturbances induced by transient ischemia of the spinal cord. Because neutrophil elastase increases vascular permeability, thereby inducing tissue ischemia,[33] it is possible that activated neutrophil-induced endothelial cell damage might lead to spinal cord ischemia after the reperfusion. Further, our preliminary study also demonstrated that PB 1.3, an anti-P-selectin monoclonal antibody that inhibits neutrophil adhesion to endothelial cells,[34] markedly inhibited the ischemia-induced spinal cord injury. These preliminary observations strongly suggest that the activated neutrophils adhering to endothelial cells might play a critical role in the endothelial cell injury by releasing various inflammatory mediators such as neutrophil elastase. Consistent with this hypothesis is a report by Clark et al[9] demonstrating that antiintercellular adhesion molecule-1 antibody reduces neurologic deficits in an animal model of spinal cord ischemia.

APC has been shown to inhibit neutrophil activation by inhibiting TNF-α production in rats given lipopolysaccharide.[13,14] The mechanism by which APC inhibits TNF-α production by activated monocytes is not well understood. Grey et al[18] demonstrated that APC suppressed the produc-

tion of TNF-α by lipopolysaccharide-stimulated monocytes by inhibiting the coupling of lipopolysaccharide and CD14, which stimulates production of cytokines, and this suppressive activity of APC is dependent on its serine protease activity. Consistent with this observation, APC attenuated the transient ischemia-induced increases in tissue levels of TNF-α and rat interleukin-8 and the subsequent neutrophil accumulation in the spinal cord tissue of rats in the present study. Because neither DEGR-F.Xa nor DIP-APC inhibited the increases in the tissue levels of these cytokines or the neutrophil accumulation in the ischemic spinal cord, APC appeared to inhibit these increases not by its anticoagulant effects, but by its serine protease activity. These observations strongly suggest that APC reduced the transient ischemia-induced spinal cord injury by inhibiting TNF-α production and consequently inhibiting neutrophil activation.

Alternatively, Grinnell et al[35] demonstrated that APC inhibits the endothelial adhesion of neutrophils by inhibiting the interaction of E-selectin with the sialyl Lewis^x antigen of neutrophils, suggesting that APC might inhibit neutrophil activation directly. However, because this inhibitory activity of APC is not dependent on its serine protease activity, the inhibition of neutrophil adhesion by APC might not be involved in the therapeutic mechanisms in the present study.

APC inhibits TNF-α production by monocytes stimulated in vitro with lipopolysaccharide in a manner dependent on the serine protease activity of APC.[14] However, lipopolysaccharide might not be involved in the pathogenesis of ischemia/reperfusion-induced tissue injury. Oxygen free radicals play a role in the ischemia/reperfusion-induced tissue injury by stimulating monocytes to produce TNF-α.[36] Thus, it is possible that APC may inhibit oxygen free radical-induced TNF-α production by monocytes. We are investigating the mechanisms by which APC inhibits the oxygen free radical-induced TNF-α production by monocytes.

Taken together, these observations raise a possibility that APC is an agent that might be useful for preventing spinal cord injury during or after aortic aneurysm repair.

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