Poly(adenosine diphosphate ribose) polymerase inhibition modulates spinal cord dysfunction after thoracoabdominal aortic ischemia-reperfusion

Patrick J. Casey, MD, a James H. Black, MD, a Csaba Szabo, MD, PhD, a Matthew Frosch, MD, PhD, b Hassan Albadawi, MD, a Min Chen, MD, PhD, a Richard P. Cambria, MD, a and Michael T. Watkins, MD, a,b Boston and Beverly, Mass

Objective: Spinal cord injury (SCI) remains a source of morbidity after thoracoabdominal aortic reconstruction. These studies were designed to determine whether PJ34, a novel ultrapotent inhibitor of the nuclear enzyme poly(adenosine diphosphate ribose) polymerase (PARP) could modulate neurologic injury after thoracic aortic ischemia reperfusion (TAR) in a murine model of SCI.

Methods: Forty-one anesthetized male mice were subject to thoracic aortic occlusion (11 minutes) through a cervical mediastinotomy followed by 48 hours of reperfusion (TAR) under normothermic conditions. PJ34-treated mice (PJ, n = 12) were given 10 mg/kg PJ34 intraperitoneally 1 hour before ischemia and 1 hour after unclamping. The control group (UN, n = 21) received normal saline intraperitoneally 1 hour before ischemia and 1 hour after unclamping. Sham animals (n = 10) were subject to thoracic aortic exposure with no aortic clamping and similar intraperitoneal normal saline injections. PARP-1−/− (KO, n = 8) mice were subjected to the same conditions as the UN mice. Blinded observers rated murine neurologic status after TAR by using an established rodent paralysis scoring system. Murine spinal cords were subjected to cytokine (GRO-1) protein analysis as a marker of inflammation and immunohistochemical analysis (hematoxylin-eosin and PAR staining). Paralysis scores (PS) and GRO-1 levels were compared with analysis of variance, and survival data were compared with χ².

Results: Immediately after TAR, UN and PJ mice had severe neurologic dysfunction (PS = 5.8 ± 0.1 and 4.6 ± 0.6, respectively; P < .05), which was significantly worse than the KO mice (PS = 1.0 ± 0.7, P < .001). After 6, 24, and 48 hours KO mice had no discernable neurologic injury (PS = 0). Six hours after TAR, PJ mice significantly improved (PS = 1.1 ± 0.73, P < .001) and remained improved at 24 (PS = 0.7 ± 0.6) and 48 hours (PS = 0.6 ± 0.6). UN mice did not improve their PS, and Sham mice showed no neurologic abnormality at any time during these experiments. The mortality at 48 hours was 0% for KO and UN mice, 43% for UN (P < .012), and 0% for Sham. KO mice were significantly decreased in PJ and KO versus UN mice (UN, 583 ± 119 vs PJ, 5.8 ± 0 vs KO, 5.3 ± 1.4 mg/pgg; P < .001). Immunohistochemistry showed evidence of decreased PAR staining and ventral motor neuron injury in PJ mice.

Conclusions: Genetic deletion of PARP or inhibition of its activity (PJ34) rescued neurologic function in mice subjected to TAR. PARP inhibition might represent a novel therapeutic approach for prevention of SCI after TAR. (J Vasc Surg 2005;41:99-107.)

Clinical relevance: Because paralysis caused by spinal cord ischemia remains a serious complication of thoracic aortic aneurysm repair, effective therapeutic strategies to prevent or decrease its incidence are desirable. Although there is hope that the systemic complications associated with open thoracic aortic aneurysm repair will be avoided by managing these lesions with catheter-based endovascular approaches, the initial experiences suggest that spinal cord ischemia might continue to be an unresolved clinical problem.

Spinal cord injury (SCI) remains a source of morbidity after thoracoabdominal aortic reconstruction. These stud-
A common result of PARP activation is cellular dysfunction leading to necrosis; however, the activation of the PARP enzyme has also been implicated in the development of apoptosis. Most PARP inhibitors act as competitive inhibitors of the catalytic domain of the enzyme, although some benzamides have been shown to exert additional effects such as inhibition of the binding of PARP to DNA. Even though there are many different PARP isoforms, the issue of selectivity has not been a problem because the active catalytic center of PARP is highly conserved. PARP inhibitors fall into the categories of monoaryl amides and bicyclic, tricyclic, or tetracyclic lactams. An inherent disadvantage of these planar heteroaromatic compounds is their poor solubility in water and organic solvents. Recent modifications of the tricyclic 1,8-naphthalimide derivatives and (5H)-phenanthridin-6-ones have resulted in the development of PJ34, a potent, water soluble, orally bioavailable with marked in vivo activity. PJ34 and related compounds inhibit PARP activation in whole blood cell based assays in the concentration range of 10 nmol/L to 1 μmol/L, with a median effective concentration in the 100 to 300 nmol/L range. They exert their in vivo anti-inflammatory and anti-reperfusion actions in a dose range of 3 to 30 mg/kg. The solubility of PJ34 in saline, its intraperitoneal absorption, and its ability to effectively inhibit PARP in biologic systems at dosages in the low milligram per kilogram dosing range made this compound an attractive agent for study in this model. Many investigations have shown that the tissue protective effect of the novel, ultrapotent PARP inhibitor PJ34 correlates with its ability to decrease PARP activity in vivo.

Despite considerable advances during the past decade, SCI complicating extensive thoracic aortic aneurysm surgery remains an unsolved problem. The multitude of adjuncts and operative strategies reviewed elsewhere directed against SCI is testimony to the fact that there remains no consensus on the optimal strategy to prevent this complication. The widespread distribution of the PARP enzyme, combined with its proven protective effects on a multitude of organs, makes PARP inhibition an attractive candidate for potential prevention of SCI in thoracoabdominal surgery. This study was designed to test the hypothesis that PARP inhibition would limit the incidence of SCI in a mouse model of thoracic aortic ischemia reperfusion.

METHODS

Experimental preparation. To achieve thoracic aortic ischemia and reperfusion (TAR), male 129S-Adprt1tm1Zqw mice (PARP-1 −/−) and their control strain 129S1/SvImJ (both strains from Jackson Laboratory, Bar Harbor, Maine), age 8 weeks and weighing 28 to 30 g, were used for these experiments. Animal care and experimental procedures complied with “Principles of Laboratory Animal Care” (Guide for the Care and Use of Laboratory Animals, National Institutes of Health publication 86-23, 1985) and were approved by the Massachusetts General Hospital animal studies committee. Anesthesia was administered with an intraperitoneal injection of sodium pentobarbital (75 mg/kg). Five units of heparin (170 IU/kg) was injected subcutaneously 5 minutes before the procedure. Temperature was maintained at 37.5°C ± 0.5°C, and animals were allowed to breathe room air throughout the experiment. TAR was achieved through a cervical mediastinotomy slightly modified from a technique initially described by Lang-Lazdunski et al. Modifications of the protocol used by Lang-Lazdunski et al include use of intraperitoneal pentobarbital anesthesia rather than inhaled halothane to facilitate positioning for noninvasive laser Doppler imaging. Qualitative confirmation of lower extremity hypoperfusion after aortic clamping and reperfusion after removal of the clamp was obtained by using laser Doppler imaging. The thoracic aorta was occluded for 11 minutes as described by Lang-Lazdunski et al. Control strain mice were assigned to study groups as follows: treated animals (PJ, n = 12) were administered the PARP inhibitor N-(6-oxo-5,6-dihydrophenanthridin-2-yl)-2-(N,N-dimethylamino)acetamide (PJ34; Calbiochem, Spring Valley, Calif) 10 mg/kg (0.3 mg in 0.3 mL normal saline) intraperitoneally 1 hour before the proce-
dure and 1 hour after the clamp was removed; untreated (UN, n = 21) and sham mice (Sham, n = 10) received vehicle (normal saline, 0.3 mL) under identical conditions; in the Sham-operated group, there was no aortic and subclavian artery occlusion. PARP +/− mice (KO, n = 8) received vehicle (normal saline, 0.3 mL) as described earlier. All experimental groups had the same volume of fluid during the perioperative period. Animals were killed at 48 hours of reperfusion with an intraperitoneal injection of sodium pentobarbital (100 mg/kg).

**Laser Doppler imaging.** To monitor lower body perfusion and to confirm TAR, laser Doppler imaging was performed. The laser Doppler scanner was mounted on a movable rack exactly 10 cm above the lower extremities and tails of the mice while the mice were restrained on a heated (37°C) operating blanket. The laser beam (780 nm), reflected from moving red blood cells in nutritional capillaries, arterioles, and venules, is detected and processed to provide a computerized, color-coded image. Mouse hind limbs and tails were scanned with a laser Doppler Imager (Moor Instruments Inc, Wilmington, Del; model LDI) immediately before, during, and after applying the aortic clamp to qualitatively document baseline perfusion and confirm ischemia followed by reperfusion. All animals that were found to have adequate hind limb and tail ischemia after aortic clamping were subjected to a complete neurologic assessment and survival analysis after reperfusion. Mice that had evidence of ongoing perfusion after aortic clamping were immediately killed and not subjected to further experimental analysis.

**Paralysis scoring scale.** Mouse hind limb neurologic function was assessed by blinded observers on awakening, at 6 hours, 24 hours, and 48 hours. Motor functions were graded by using a mouse paralysis score (PS) system16: (1) walking with hind limbs: 0, normal; 1, toes flat under body when walking but ataxia is present; 2, knuckle-walking; 3, movements in hind limbs but unable to walk; and 4, no movement, drags hind limbs; and (2) placing/stepping reflex: 0, normal; 1, weak; and 2, no stepping. Each grade was obtained by adding the scores for (1) and (2) to provide the PS.

**Histopathology.** Animals were killed at 48 hours of reperfusion as described above. The entire spine was harvested en bloc and fixed in 10% buffered formalin for 24 hours. The cords were transferred to 70% alcohol. Specimens were decalcified for 1 week. After paraffin embedding, thin sections of cord were prepared from the thoracolumbar cord (T8-L5) for immunohistochemical detection of poly(ADP ribose) formation, an immunohistochemical indicator of the activation of PARP and examination under hematoxylin-eosin (fast blue hematoxylin-eosin; Biomedia, Foster City, Calif). Immunohistochemical detection of poly(ADP ribose) was performed in paraffin sections (3 μm) after deparaffinization in xylene and rehydrated in decreasing concentrations (100%, 95%, and 75%) of ethanol followed by a 10-minute incubation in phosphate-buffered saline (PBS) (pH, 7.4). Sections were treated with 0.3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity and then rinsed briefly in PBS. Nonspecific binding was blocked by incubating the slides for 1 hour in 0.25% Triton/PBS containing 2% horse serum. Poly(ADP ribose) was detected by using a chicken monoclonal anti-poly(ADP ribose) antibody (Tulip Biolabs, West Point, Pa) and isotype-matched control antibody, applied in a dilution of 1:250 for 1 hour at room temperature. After extensive washing (3 × 10 min) with 0.25% Triton/PBS, immunoreactivity was detected with a biotinylated horse anti-chicken secondary antibody and the avidin-biotin-peroxidase complex, both supplied in the Vector Elite kit (Vector Laboratories, Burlingame, Calif). Color was developed by using Ni-DAB substrate-kit (Vector Laboratories). Sections were then counterstained with nuclear fast red, dehydrated, and mounted in Permount.17 Photomicrographs were taken with a Zeiss Axioslab microscope (Zeiss Microscopy, Welwyn Garden City, United Kingdom) equipped with a Fuji HC-300C digital camera (Fuji Film Medical Systems, Stamford, Conn). All immunohistochemical samples were coded and examined and graded by an investigator in a blinded fashion.

**Cord protein analysis.** Protein samples were extracted from the ischemic zone (T8-L5) of representative spinal cords for subsequent cytokine analysis. Excised tissue was immediately frozen, crushed into powder in liquid N2, and then homogenized on ice (protease cocktail) and centrifuged at 11,000 × g for 30 minutes at 4°C.18 Supernatants from these extracts were analyzed for GRO-1 by using enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, Minn). The results were normalized to total protein in each extract.

**Statistical analysis.** Survival data were calculated by using Fisher exact t analysis at 6, 24, and 48 hours. Neurologic score and GRO-1 levels between treatment groups at each experimental interval were calculated by using analysis of variance (ANOVA) (Kruskal-Wallis test, nonparametric ANOVA). Post hoc analysis included the Dunn multiple comparison test.

**RESULTS**

**Survival.** Mortality (overall 43%) was observed only in the control, untreated mice subjected to TAR. Two UN mice died while the clamp was on. Four UN mice died after awakening but before the 6-hour evaluation interval. At the 6- and 24-hour intervals, there was no significant difference between the survival of the UN and PJ mice (6 hours, P = .522; 24 hours, P = .585); two of the three mice dying between 24 and 48 hours were noted to have hematuria and mesenteric infarction at necropsy. No Sham or PJ animal (n = 12) died before or after the clamps were removed. All PARP +/− mice (n = 8) survived the TAR throughout the entire 48-hour period. At necropsy, no PJ or PARP −/− mouse had gross evidence of hematuria or ischemia/necrosis disease in the visceral organs. When compared to untreated animals, PJ (P = .0050, χ²) and KO mice (P = .0265, χ²) had significantly better survival at 48 hours.

**Paralysis scoring.** The results of the neurologic injury scoring are represented in Fig 2. All surviving UN and PJ mice awoke with significant neurologic dysfunction (PS =
The different morphologic appearances of the thoracolumbar cord in UN, KO, and PJ mice are demonstrated in Fig 5. In the outlined section of the anterior horns in the UN mice, there is evidence of vacuolization and a paucity of motor nuclear cells. There is preservation of the anterior motor neuron complex in the PJ and KO mice. Immunohistochemical staining for poly(ADP ribose), the catalytic product of the PARP enzyme, revealed that there was marked activation of the enzyme in the few identifiable anterior motor nuclear cells visible after thoracic aortic ischemia-reperfusion (Fig 6, C). Compared to the low basal PARP activity detected in the Sham controls (Fig 6, A), there were very few intact anterior motor nuclei in the cords of UN mice (Fig 6, C). The ability of PJ34 to inhibit the catalytic activity of the PARP enzyme in vivo was shown by minimal poly(ADP ribose) immunostaining seen in the inhibitor treated animals (Fig 6, B), along with intact anterior motor nuclei.

Spinal cord cytokine levels. UN mice were found to have markedly elevated GRO-1 levels (583 ± 119, P < .0001) versus Sham (6.1 ± 0.2 mg/pg cord protein, n = 8), KO (5.8 ± 1.0 mg/pg, n = 6), and PJ mice (5.3 ± 1.4 mg/pg, n = 6). Sham, PJ, and KO mice had extremely low levels of GRO-1, which were not statistically different from one another.

DISCUSSION

These experiments indicate that the PARP inhibitor PJ34 preserves neurologic function and survival in a murine model of TAR. The benefit of PARP inhibition was verified by using an approach of genetic deletion of this important nuclear enzyme. The use of transgenic mice lacking the functional PARP enzyme has provided unequivocal data to support its role in myocardial ischemia-reperfusion injury, and as such provides rationale for a transgenic approach to studying neurologic dysfunction after TAR. This report is among the first to apply specific genetic deletion to examine therapeutic strategies for prevention of spinal cord ischemia-reperfusion. The salvage of neurologic function by PARP inhibition with PJ34 and PARP genetic deletion was statistically evident after awakening and remained evident throughout reperfusion (Fig 2). The reason for pursuing PARP inhibition to limit spinal cord ischemia was based on successful interventions seen in animal models of stroke, endotoxic shock, and myocardial ischemia. Unlike other inhibitors of PARP, PJ34 is water soluble and can be diluted in saline (which has no antioxidant capacity). Other potent inhibitors of PARP must be solubilized in dimethyl sulfoxide, which can scavenge reactive oxygen metabolites and possibly complicate experimental analysis. The dosage of PJ34 used in these experiments paralleled pre-injury and postinjury regimen used for intravenous administration of PJ34 in rodent model of stroke, endotoxemia/gut ischemia, and sepsis. Combined pretreatment and posttreatment regimen was favored for these experiments because of the severe systemic stress associated with TAR, the short half-life of PJ34 in plasma (30-minute half-life), and because the intraperitoneal route of administration might...
have provided less uniform systemic absorption of the drug. Although murine neurologic function was significantly improved immediately after TAR in KO and PJ mice, a survival advantage with respect to PJ34 treatment was not clearly evident until 48 hours. In treated animals and KO mice, there were no deaths. In contrast, there were six early deaths, i.e., expiration with the clamp on or within 6 hours of thoracic aortic occlusion in UN animals. A possible explanation for the trend toward decreased early mortality in KO mice and mice treated with PARP inhibitors might be based on the physiology of thoracic aortic occlusion. Activation of PARP during thoracic aortic occlusion might contribute to myocardial ischemia prompted by increased myocardial oxygen demand caused by increased afterload. We did not specifically evaluate myocardial PARP activation in these experiments. However, Faro et al have documented PARP activation in porcine myocardium after brief periods of ischemia-reperfusion and have shown that treatment with PJ34 effectively ameliorates the physiologic consequences of its activation. In addition to the effects of proximal aortic occlusion on myocardial oxygen demand, the choice of pentobarbital for anesthesia and room air for ventilation might have also influenced early mortality surrounding mice subjected to TAR in the absence of PJ34 treatment. The early mortality rate reported by Lang-Lazdunski et al was 21%, whereas the mortality observed in these experiments was 43%. We chose pentobarbital and room air over halothane and 100% oxygen to facilitate laser Doppler imaging. Both pentobarbital and halothane have been reported to have protective effects toward spinal cord ischemia. Laser Doppler imaging was used to document lower torso ischemia in each mouse subjected to TAR. We identified eight mice that were ostensibly occluded by clamps at the thoracic arch but had visual evidence of ongoing lower torso perfusion on laser Doppler imaging. The ongoing distal perfusion observed in the setting of thoracic aortic cross-clamping might have been related to microvascular clamp fatigue, clamp position, or large collateral pathways through the anterior abdominal wall. To assure consistency in the degree of ischemia for each mouse included in these experiments, no attempts were made to readjust the clamps to re-establish torso ischemia in these mice. Thus the elimination of these mice from the study and subsequent neurologic analysis provided objective confirmation that thoracic aortic ischemia was indeed a common factor in all mice subjected to reperfusion. This is in contrast to the work of Lang-Lazdunski et al, who documented decreased cord perfusion by using invasive monitoring only in a subset of mice not subjected to further neurologic or survival analysis.

Three of the UN mice died after 24 hours of recovery. Necropsy showed that mesenteric ischemia might have contributed to two of these late deaths. Prolonged mesenteric ischemia has been correlated with increased inflammatory response after thoracic aortic aneurysm repair and has provided the impetus for mesenteric shunting during com-

Fig 3. Cross-sectional spinal cord anatomy of an untreated mouse (original magnification, x10). Forty-eight hours after TAR, global depletion of anterior motor neuron cells (A) with preservation of the sensory posterior columns (P) is demonstrated in this photomicrograph of the thoracolumbar spine. The dorsal sensory nuclei were preserved (D).
plex cases. The incidence of early perioperative deaths and autopsy findings of mesenteric infarction strongly suggest that the murine model might be more robust and relevant for study of the complex systemic complications of TAR in addition to spinal cord ischemia.

Although the findings in the current report parallel the effect of other pharmacologic agents in a variety of animal models, the murine model used might have more relevance to the human condition. It is obvious that the murine model is useful because it is suited for molecular manipulations with specific knockout (ie, genetic deletions) and transgenic mice. This model might be more relevant to the human condition because the blood supply to the murine spinal cord is nonsegmental. Humans experience a unique type of spinal cord dysfunction, ie, anterior cord syndrome after ischemia-reperfusion injury. In human cord ischemia-reperfusion injury, the anterior motor neurons are damaged, and the posterior columns are spared. The vertical orientation of the blood supply to the human spinal cord (single anterior and dual posterior

Fig 4. High power photomicrographs of the thoracolumbar spinal cord (original magnification, ×40). A, Evidence of chromalysic Nissl bodies in an anterior motor neuron of an untreated mouse 48 hr after TAR (black solid arrows). B, Evidence of preserved motor nuclei in a Sham animal (white solid arrows). C, Axonal spherocytosis in an untreated animal 48 hr after TAR (dotted black arrows).

Fig 5. Distal thoracic spinal cord photomicrographs (original magnification, ×10). Untreated mice show widespread depletion of anterior motor neuron cells, whereas PJ and PARP −/− (KO) mice exhibit preserved anterior motor neurons.
spinal arteries) is thought to be responsible in part for preserving the posterior sensory columns at the expense of the anterior motor neurons. In contrast, the blood supply to pigs and rabbits is segmental and horizontal. In the pig and rabbit model of ischemia-reperfusion, there is simultaneous motor and sensory defect at the level of aortic clamping. In the experiments reported in this article, histologic examination of the spinal cords of untreated animals revealed evidence that the posterior (or dorsal sensory) elements of the cords were preserved after thoracic aortic occlusion (Fig 3), consistent with the clinical finding in human patients afflicted with spinal cord ischemia after thoracic aortic ischemia-reperfusion. PJ mice showed no evidence of ventral motor neuronal injury (Figs 4 and 5). PJ mice also showed minimal evidence of PAR immunohistochemistry murine spinal cord at 48 hr (original magnification, ×40). A, Sham mouse cord demonstrating many anterior motor neurons and no intense PAR staining. B, PJ mouse with abundant anterior motor nuclei and sparse PAR staining. C, Untreated mouse cord demonstrating few anterior motor nuclei and focal intense PAR staining.

tissue necrosis. Detailed immunohistochemical analysis suggests that microglia cells, not peripheral inflammatory cells, are the main source for cytokine and chemokines in the central nervous system. Acute central nervous system injury has been associated with transient release of inflammatory mediators such as interleukin-1 and tumor necrosis factor-α during the first few hours of ischemia. In contrast to the cytokine profile during acute central nervous system injury, GRO-1, also known as KC, the murine equivalent of human interleukin-8, is a CXC chemokine implicated in chronic inflammatory processes within the central nervous system. GRO-1 was markedly increased in the spinal cord tissue of UN mice, as compared to Sham, KO, and PJ mice. This finding indicates that PARP inhibition or genetic deletion successfully decreases the cytokine response in the spinal cord after TAR. Although knockout mice have been used to delineate a role for Tissue Plasmin-

Injury to the spinal cord is complicated by inflammatory mechanisms mediated by influx of monocytes and macrophages after the secretion of cytokines and chemokines. Detailed immunohistochemical analysis suggests that microglia cells, not peripheral inflammatory cells, are the main source for cytokine and chemokines in the central nervous system. Acute central nervous system injury has been associated with transient release of inflammatory mediators such as interleukin-1 and tumor necrosis factor-α during the first few hours of ischemia. In contrast to the cytokine profile during acute central nervous system injury, GRO-1, also known as KC, the murine equivalent of human interleukin-8, is a CXC chemokine implicated in chronic inflammatory processes within the central nervous system. GRO-1 was markedly increased in the spinal cord tissue of UN mice, as compared to Sham, KO, and PJ mice. This finding indicates that PARP inhibition or genetic deletion successfully decreases the cytokine response in the spinal cord after TAR. Although knockout mice have been used to delineate a role for Tissue Plasmin-
These experiments provide a novel perspective on the etiology of neurologic dysfunction after TAR, firmly implicating a role for the DNA repair enzyme PARP in the etiology of SCI. On the basis of what is known about PARP consumption of ATP and NAD⁺ during energy crises, we suspect that PJ34 decreases intracellular energy depletion in spinal cord tissues during TAR, which might decrease cellular stress during ischemia-reperfusion, resulting in decreased inflammatory response. The double-edged sword of PARP activity suggests that its inhibition in a number of clinical conditions such as arthritis, sepsis, diabetes, stroke, inflammatory bowel disease, and spinal cord ischemia might provide a useful venue for intervention in human diseases. Before potent PARP inhibitors can be used in humans, crucial safety issues must be addressed. PARP has been implicated in DNA repair and maintenance of genomic integrity; therefore one possible risk associated with long-term PARP inhibition might be increased mutation rate, possibly associated with cancer formation. PARP-deficient mice have not been reported to have an increased occurrence of spontaneous tumors; however, increased incidences of chemically induced tumors have been identified in these mice.³ Issues such as cancer risk are largely mitigated by short-term treatment with PARP inhibitors in acute life-threatening indications such as stroke, myocardial ischemia, and repair of thoracic aortic aneurysms.

Because paralysis caused by spinal cord ischemia remains a serious complication of thoracic aortic aneurysm repair, effective therapeutic strategies to prevent or decrease its incidence are desirable. Although there is hope that the systemic complications associated with open thoracic aortic aneurysm repair will be avoided by managing these lesions with catheter-based endovascular approaches, the initial experiences suggest that spinal cord ischemia might continue to be an unresolved clinical problem.⁸

REFERENCES

29. Andrews DM, Matthews VB, Sammels LM, Carrello AC, McMinn PC. The severity of murray valley encephalitis in mice is linked to neutrophil


