

# Comparison of Systemic and Retrograde Delivery of Adenosine A<sub>2A</sub> Agonist for Attenuation of Spinal Cord Injury After Thoracic Aortic Cross-Clamping

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**Background.** Paraplegia remains a devastating complication of thoracic aortic surgery, which has been attenuated by retrograde adenosine and systemic adenosine A<sub>2A</sub> receptor activation. We hypothesized that despite retrograde spinal perfusion of an adenosine A<sub>2A</sub> agonist (ATL-146e), systemic therapy produces superior spinal cord protection with reduced inflammation.

**Methods.** Forty pigs underwent 30-minute thoracic aortic cross-clamping. Pigs received: no therapy (control); retrograde saline (retrograde control); retrograde ATL-146e; systemic ATL-146e; systemic ATL-146e with retrograde saline; or systemic and retrograde ATL-146e. Retrograde therapies were given during ischemia. Systemic ATL-146e (0.06 μg · kg<sup>-1</sup> · min<sup>-1</sup>) was given intravenously for 3 hours at reperfusion. At 24 hours, motor function was assessed using the Tarlov scale. Tissue was analyzed for neuronal viability, microtubule-associated protein-2 expression, and neutrophil sequestration (myeloperoxidase activity).

**Results.** Four pigs received retrograde barium showing both radiographic and histologic spinal cord perfusion.

Tarlov scores at 24 hours were significantly improved versus both control groups in all ATL groups except the combined ATL-146e group (all  $p < 0.05$ ). Neuronal viability by hematoxylin and eosin stain was significantly preserved in systemic ATL groups compared with both control groups (all  $p < 0.05$ ). Microtubule-associated protein-2 expression was significantly preserved compared with both control groups in all systemic ATL groups. Systemic ATL significantly lowered myeloperoxidase activity versus both control groups ( $p < 0.01$ ).

**Conclusions.** Both retrograde and systemic ATL-146e therapies attenuate ischemic spinal cord injury, but combining the two routes was less effective. Given comparable results between the two routes and the simplicity of systemic delivery, peripheral venous ATL-146e at reperfusion should be preferred for spinal cord protection in thoracic aortic surgery.

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Despite the emergence of perioperative protective strategies, paraplegia after thoracoabdominal aortic intervention still occurs in 1.5% to 16% of cases [1–5]. A plethora of substances have been shown to attenuate ischemic spinal cord injury, but pharmacologic protection has not produced results sufficient to warrant widespread use in these cases [4, 6–9]. Adenosine A<sub>2A</sub> receptor activation may change this trend in the future as an adjunct to further prevent spinal cord injuries in combination with current strategies.

Previous studies have demonstrated that adenosine administered in a retrograde fashion during ischemia preserved spinal cord function after reperfusion of an ischemic insult [10–12]. However, adenosine use re-

quires regional hypothermic delivery secondary to systemic cardiovascular effects and to rapid metabolism at normal body temperature. These obstacles have limited the implementation of adenosine in patients. More recently, specific adenosine receptors have been elucidated. One of these specific receptors may be responsible for the protective actions of adenosine after reperfusion of an ischemic insult [13]. Activation of the adenosine A<sub>2A</sub> receptor, using the substance ATL-146e, at reperfusion has been shown to reduce ischemia-reperfusion injury in the heart, lung, and kidney [14–16]. The substance ATL-146e has also been neuroprotective in the rabbit models of both spinal

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Doctors Kron and Linden disclose that they have a financial relationship with Adenosine Therapeutics, LLC.

cord trauma and spinal cord ischemia in terms of motor function, neuronal apoptosis, and systemic cytokine response to injury [17-19]. These earlier studies suggest that ATL-146e is efficacious when given systemically, but regional delivery, similar to the adenosine studies, has not been tested.

This study aimed to compare delivery routes of ATL-146e: regional retrograde delivery through the hemiazygous vein during the ischemic period versus systemic delivery through peripheral veins at reperfusion. Initially, the ability of regional route to deliver the compound to the spinal cord vasculature was determined. Next, the study compared retrograde delivery with systemic delivery of ATL-146e for spinal cord protection. The routes of delivery were compared using outcomes of function, of neuronal cytoarchitecture and, finally, with an inflammatory marker. We hypothesized that although retrograde drug delivery does reach the spinal microvasculature, systemic ATL-146e given at reperfusion will preserve spinal cord function, preserve neuronal cytoarchitecture, and decrease neutrophil sequestration better than retrograde therapies in thoracic aortic cross-clamping.

## Material and Methods

All protocols were reviewed and approved by the Animal Care and Use Committee of the University of Virginia. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals," as described by the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (Washington: National Academy Press, 1996).

Initially, four pigs (30 to 35 kg) were utilized to demonstrate retrograde delivery of fluid to the spinal cord parenchyma. Then 36 pigs (30 to 35 kg) were utilized in a swine model of spinal cord ischemia. They were randomly divided into six groups defined in the procedure section. They all underwent 30 minutes of thoracic aortic occlusion. All animals were survived for 24 hours of reperfusion, when final functional assessment was completed.

### Procedures

Domestic pigs were anesthetized using intramuscular telazol (4 to 6 mg/kg) and xylazine (1 mg/kg). The pigs were then intubated and placed in a right lateral decubitus position. A volume ventilator was connected with a respiratory rate of 14 and a tidal volume of 250 mL. Inhaled anesthesia was maintained with vaporized halothane titrated to effect. Hemodynamic parameters were monitored through ear arterial line. Ear veins were utilized for peripheral venous access. Maintenance fluids were normal saline given at a rate of 100 mL/h during the procedure and the recovery period. Temperatures were monitored with a rectal probe and maintained with a heating pad. All fluids were warmed in a circulating bath before delivery at 36°C. Heart rate and mean arterial pressure were monitored during the ischemic and recovery periods.

Table 1. Hind Limb Motor Function: Definitions of Tarlov Scores

Tarlov Score	Best Hind Limb Motor Function
0	No hind limb movement
1	Moves hind limbs
2	Sits with assistance
3	Sits alone
4	Unsteady gait
5	Normal gait

The procedure was performed through a left lateral thoracotomy. The left lung was retracted anteriorly to expose the accessory hemiazygous vein and the descending aorta. The accessory hemiazygous vein was cannulated, ligated, and transected for access to the retrograde conduit. The descending aorta was isolated circumferentially. After systemic heparinization (100 u/kg heparin sulfate) cross-clamp was placed across the aorta distal to the takeoff of the left subclavian artery.

Four pigs underwent the procedure with retrograde delivery of barium sulfate at a rate of 500 mL/h for 15 minutes under fluoroscopy. These animals were euthanized with an overdose of pentobarbital before excision lumbar spinal cord sections, which were taken for histology. The sections were observed using dark-field microscopy to demonstrate penetration of the contrast into the parenchymal vessels.

All other groups (each n = 6) survived for 24 hours after reperfusion. Ischemia-reperfusion control animals (IR) underwent 30 minutes of aortic occlusion without further therapy. Retrograde therapies through the accessory hemiazygous vein included retrograde normal saline (rNS) at a rate of 500 mL/h during ischemia, retrograde ATL-146e (rATL) at a rate of  $0.06 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in normal saline at 500 cc/h during ischemia. Systemic ATL-146e animals (sATL) received ATL-146e at a rate of  $0.06 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for 3 hours starting 10 minutes before reperfusion. Two additional groups were used to evaluate combination treatments including retrograde normal saline during ischemia at 500 mL/h with systemic ATL-146e (rNSsATL) at a rate of  $0.06 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  and both retrograde ATL-146e  $0.06 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in normal saline at 500 cc/h during ischemia and systemic ATL-146e at  $0.06 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  over the first 3 hours of reperfusion (rsATL). The dose of ATL-146e given in this study had been previously established as optimal for ischemic spinal cord protection [18].

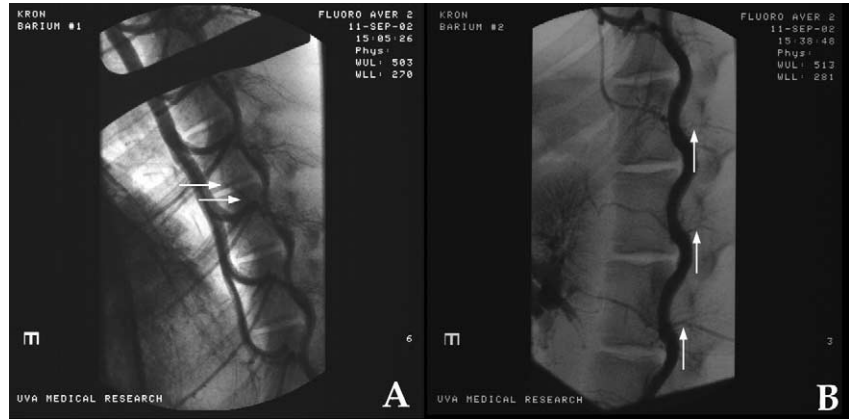
### Hind Limb Motor Function

A masked observer graded hind limb motor function using the Tarlov scale. The Tarlov scoring system is summarized in Table 1. Functional scores were taken at 12 hours and 24 hours after reperfusion.

### Spinal Cord Tissue

Twenty-four hours after reperfusion, the pigs were again anesthetized. An intravenous catheter was placed to give

Fig 1. Venograms of the (A) anterior-posterior and (B) lateral views of the chest. Both show contrast injected into the accessory hemiazygous vein, which in turn filled the paravertebral and the epidural veins. The arrows point out the epidural veins.



the euthanasia solution. The left chest was reopened. The descending aorta was excised several centimeters above and below the takeoff of subclavian artery. The aortic sample was examined to verify that the cross-clamp occluded the highest lumbar artery. Any animal with evidence of clamp placement below the highest lumbar was excluded from the study no matter their functional results. From a prone position, the back was incised and bilateral laminectomies were preformed. The lumbar spinal cord was extracted with a 1-cm section fixed in formalin for histology and a 1-cm section flash frozen for molecular studies.

### Histology

After harvest, lumbar sections of the spinal cords were processed with 10% zinc formalin fixation and routine paraffin embedding. Spinal cord sections were stained with hematoxylin and eosin. Masked observers graded randomly coded sections from all of the groups. Neuron viability was assessed by maintenance of the nuclear and cytological architecture in addition to axonal degeneration. The cells were considered viable if they had a stable nucleolus, there was minimal retraction from the surrounding tissue, and there was no evidence of axonal degeneration. Four sections from each animal were graded by the number of viable neurons in a high-powered field.

### Microtubule-Associated Protein-2 Immunohistochemistry

Embedded sections in paraffin were cut transversely with a microtome. Ten-micrometer sections were mounted on slides and deparaffinized in xylenes and progressive alcohol rinses. Microtubule-associated protein-2 (MAP-2) immunoreactivity was performed according to the previously described protocol using a murine MAP-2 antibody [20]. Reacted sections were mounted, dehydrated, and coverslipped for bright-field light microscopic examination.

The percentage of grey matter stained positively for MAP-2 within injured spinal cord was quantified and statistically compared among treatment groups and control animals. Four semiserial spinal cord sections

from each animal reacted for MAP-2 were examined with a microscope interfaced with a CCD digital camera and computer image analysis system (VisionGauge Image Analysis Software; VISIONx, Pointe Claire, Quebec). Images of spinal cord sections were captured and digitized. Grey matter was outlined in the Vision Gauge system, and background staining thresholds were calculated in adjacent white matter tissue for each section. Percentage of grey matter tissue staining positively above background thresholds were then calculated by the VisionGauge software. Mean percentage of MAP-2 staining was calculated for each animal.

### Neutrophil Sequestration

Neutrophil sequestration in the spinal cord was quantified using a myeloperoxidase assay (MPO). The MPO assay was performed according to the previously published protocol [21]. Absorbance at 460 nm was measured after 2 minutes by spectrophotometry (MRX Revelation Plate Reader; Dynex Technologies, Chantilly, Virginia). Protein concentrations were determined in each sample using the Coomassie Plus Protein Assay (BioRad, Hercules, California). Myeloperoxidase activity was then expressed as a change in absorbance per milligram of protein per minute.

### Statistics

The statistics were analyzed by our statistician using analysis of variance with Bonferroni multiple comparison tests to determine significant differences. A nonparametric analysis provided the same conclusions as the analysis of variance.

## Results

### Fluoroscopy/Dark-Field Microscopy

Evaluation of fluoroscopic images of all four animals receiving retrograde barium demonstrated filling of the epidural veins (Fig 1). Dark-field and light microscopy further showed barium in the parenchymal blood vessels of the spinal cord in all four pigs as well (Fig 2).

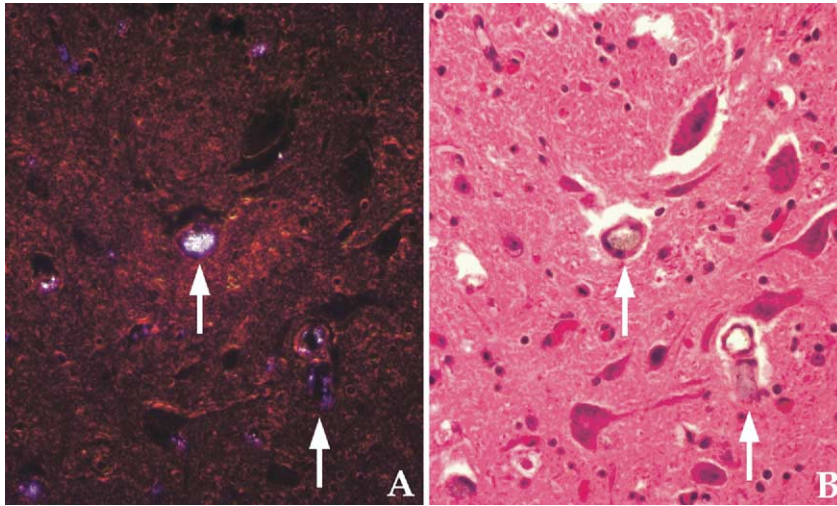


Fig 2. (A) Dark-field and (B) light microscopy of hematoxylin and eosin stained spinal cord sections demonstrating the filling of spinal cord parenchymal vasculature with barium sulfate. The white areas in the dark-field slide represent the contrast within the vessels in the tissue.

### Hemodynamics

The heart rates and mean arterial pressures were similar among all the groups during both the ischemic and recovery periods.

### Hind Limb Function

Functional assessments at 12 and 24 hours were determined. The results are depicted in Figure 3. Mean Tarlov scores recorded at 12 hours in all four groups who received ATL through retrograde or systemic delivery had better function than IR or retrograde normal saline (all  $p < .001$ ). At 24 hours, all ATL groups except rsATL

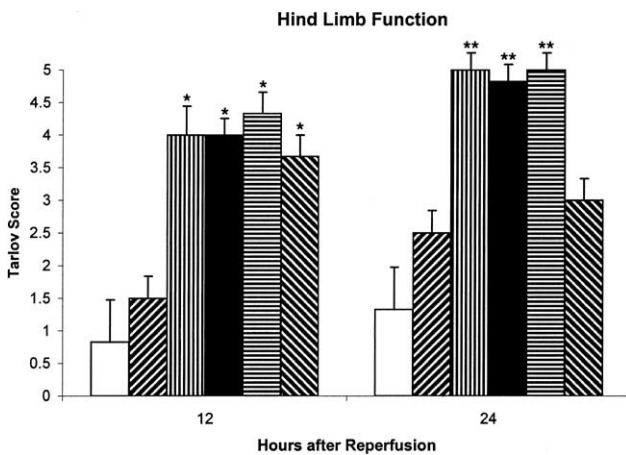


Fig 3. Comparison of neurologic function 12 hours and 24 hours after spinal cord ischemia-reperfusion. At 12 hours, function is significantly better in all groups receiving adenosine  $A_{2A}$  agonist (ATL-146e) compared with control group. At 24 hours, function is significantly better in all groups receiving ATL-146e except the group who received both systemic (s) and retrograde (r) ATL. Data are expressed as mean Tarlov score  $\pm$  SE. \* $p < 0.001$  compared with control and retrograde normal saline (NS) group. \*\* $p < 0.001$  compared with control, rNS, and rsATL groups. (Control = open bar; rATL = vertical striped bar; rNS = right-hatched bar; rNSsATL = horizontal striped bar; rsATL = left-hatched bar; sATL = solid bar.)

had significantly preserved function compared with both IR and retrograde normal saline ( $p < 0.001$ ).

### Neuronal Cytoarchitecture

Neuronal viability was counted as the number of viable neurons per high-powered field (Fig 4). Systemic ATL-146e with and without retrograde normal saline were significantly better than ischemia only and retrograde plus systemic ATL (all  $p < 0.05$ ). Expression of MAP-2 was significantly preserved in all the groups receiving systemic ATL were significantly better than the groups who did not ( $p < 0.01$ , Figs 5 and 6).

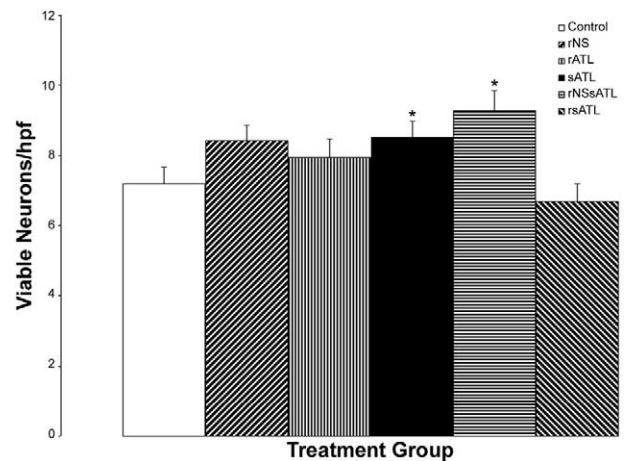


Fig 4. Neuronal viability expressed as number of neurons per high-powered field. Systemic (s) adenosine  $A_{2A}$  agonist (ATL) with and without retrograde (r) normal saline (NS) preserved neuronal integrity significantly better than control and the group receiving systemic and retrograde normal saline. Data are expressed as mean  $\pm$  SE. \* $p < 0.05$  compared with control and rsATL. (Control = open bar; rATL = vertical striped bar; rNS = right-hatched bar; rNSsATL = horizontal striped bar; rsATL = left-hatched bar; sATL = solid bar.)

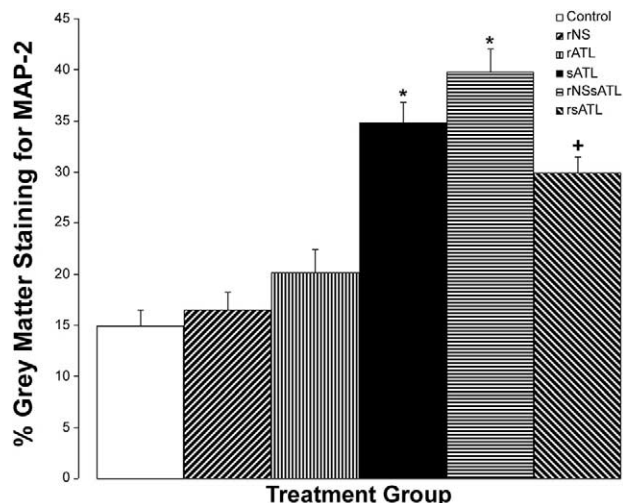


Fig 5. Microtubule-associated protein-2 (MAP-2) expressed as percent gray matter staining per section. All groups receiving systemic adenosine  $A_{2A}$  agonist (ATL) therapies preserved MAP-2 staining significantly better than those not receiving systemic ATL. Data are expressed as mean percentage expression of MAP-2  $\pm$  SE. \* $p < 0.01$  compared with control, rNS, and rATL. † $p < 0.01$  compared with control and rNS, and  $p = 0.03$  versus rATL and rNSsATL. (NS = normal saline; r = retrograde; s = systemic; control = open bar; rATL = vertical striped bar; rNS = right-hatched bar; rNSsATL = horizontal striped bar; rsATL = left-hatched bar; sATL = solid bar.)

### Myeloperoxidase Assay

Results for the MPO assay ( $\Delta OD \cdot mg^{-1} \cdot min^{-1}$ ) are shown in Figure 7. The values were significantly less in all groups receiving systemic ATL-146e compared with IR (all  $p < 0.01$ ). This included sATL, rNSsATL, and rsATL. Retrograde ATL or normal saline alone had no significant effect on neutrophil sequestration when compared with IR as demonstrated by MPO activity.

### Comment

Temporary occlusion of the thoracic aorta can carry a significant risk of paraplegia, especially in prolonged procedures. Protective strategies have included selective vascular perfusion, lumbar cerebrospinal fluid drainage, left heart bypass, and regional cooling [1-5]. Although these techniques may reduce rates of paraplegia, some risk of spinal cord injury remains after these procedures. Pharmacologic intervention has failed to date to show results that were sufficiently promising to be applied to patient care in this setting. Despite the failure of previous pharmacologic intervention, we believe that adenosine  $A_{2A}$  receptor activation has the potential to serve as a protective adjunct to the current protective strategies for at-risk vascular procedures.

Prior studies using the swine model utilized in this study have demonstrated that retrograde hypothermic adenosine prevented or lessened spinal cord ischemia-reperfusion injury caused by aortic cross-clamping [11, 12]. Two separate mechanisms were concluded to impact the local protection of spinal cord function. Cooling of the

spinal cord alone produced a significant effect that was further attenuated by the addition of adenosine. Despite positive results of neuroprotection with retrograde adenosine, the proponents of adenosine, including the authors of these studies, do not utilize this substance in their patients. Adenosine's limitations include arguments that hypothermic delivery could lead to coagulopathy and that additional procedures were required to deliver the fluid regionally to avoid the systemic degradation of the drug.

Furthermore, retrograde delivery has instigated some controversy over the local effects of various therapies [22]. Although the regional delivery of the adenosine was supported by the filling of the epidural veins, there was no direct evidence of delivery to the spinal cord vasculature. Venous stasis from aortic cross-clamping should increase retrograde perfusion of the spinal cord. The current study was able to show that retrograde venous delivery would fill both the epidural veins and that this would penetrate the parenchymal vessels. Although these findings imply that the retrograde route does reach the spinal cord parenchyma, the barium is large to extravasate into the spinal parenchyma. Despite the lack of extravasation into the tissue, this is evidence for the local vascular delivery of therapies to the spinal cord through the accessory hemiazygous vein.

More recent adenosine study has focused on the activation of the adenosine  $A_{2A}$  receptor subtype being responsible for the protective effects of adenosine in ischemia-reperfusion injury. Using the adenosine  $A_{2A}$  receptor agonist ATL-146e, we have previously presented improved outcomes after rabbit spinal cord ischemia-reperfusion. Function was improved, while neuronal cytoarchitecture was preserved in rabbits receiving the drug compared with ischemic controls [23]. The results in this initial study were accomplished without the systemic side effects of adenosine, specifically, without hypotension or bradycardia. The current study corroborates this finding without evidence of differences in hemodynamics among groups. The current study intended to show that protection from ischemia-reperfusion injury in the spinal cord was superior with systemic therapy compared with retrograde therapy delivered during the ischemic period. We found that systemic therapy at reperfusion produced significant preservation of functional outcomes compared with ischemic controls. Retrograde ATL therapies also significantly improved function compared with controls. No significant differences could be directly identified among the ATL groups, but systemic delivery required nothing more than peripheral venous access. For this reason, we believe that systemic delivery at reperfusion should be preferred over retrograde delivery during ischemia.

The animals receiving ATL-146e by combined routes functionally deteriorated significantly; and they had significantly lower neuronal viability than the other two groups who received systemic ATL-146e. This group was also the only group in the study that showed a functional decline over the observation period. The significance of these findings is not known at this time, but requires

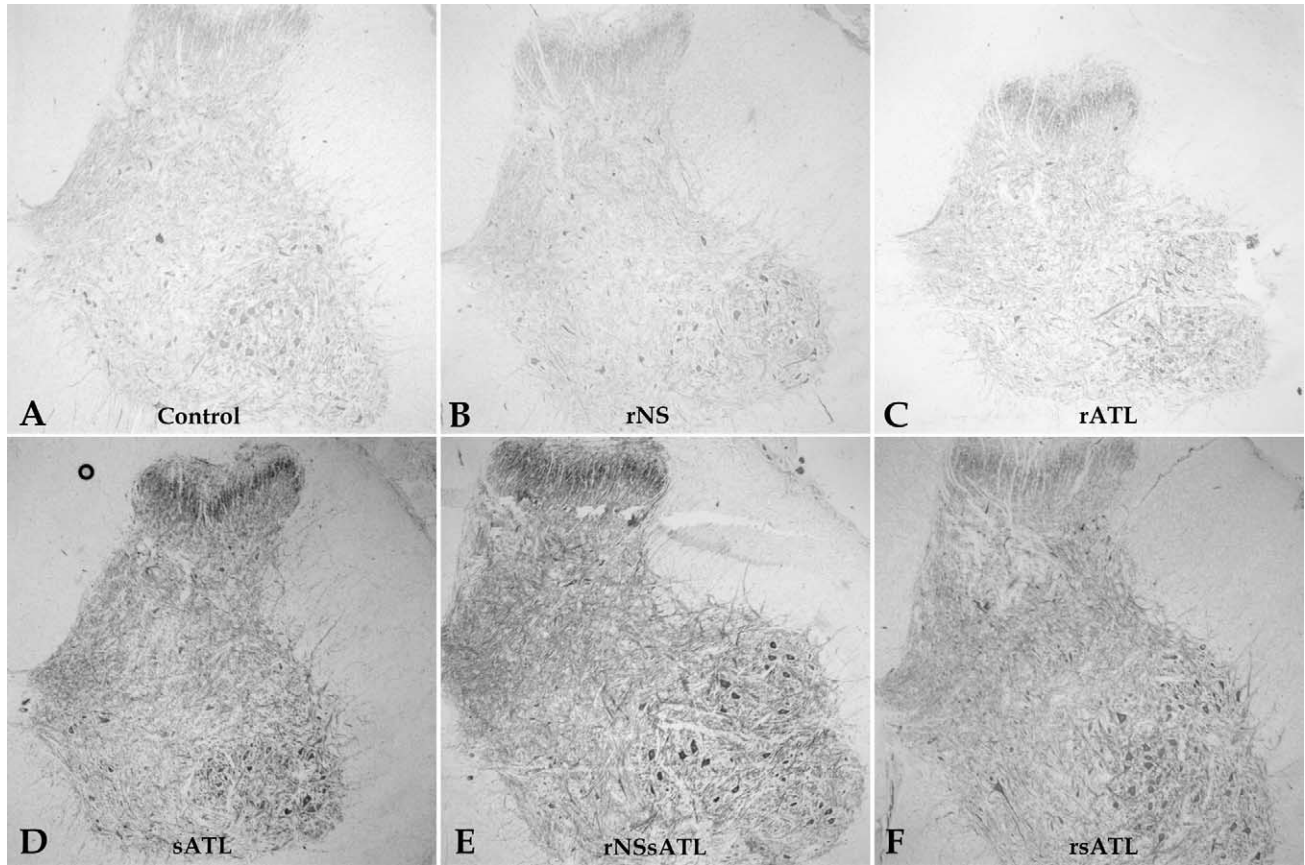


Fig 6. Microtubule-associated protein-2 (MAP-2) expressed as percent gray matter staining per section. All groups receiving systemic adenosine  $A_{2A}$  agonist (ATL) therapies preserved MAP-2 staining significantly better than those not receiving systemic ATL. (A-F) The relative expression of MAP-2 is depicted for each of the groups, with the darker staining indicating the presence of more of the protein. (NS = normal saline; r = retrograde; s = systemic.)

further study. The possibility remains that the protective mechanism of adenosine  $A_{2A}$  receptor activation differs depending on the time of delivery. Adenosine may play a role in ischemic preconditioning effects, which may be responsible for the attenuation of the injury when given during the ischemic period. Further adenosine  $A_{2A}$  activation during reperfusion after being given during ischemia may actually exacerbate the injury.

Overall, the functional outcomes after 24 hours of reperfusion appeared to mirror neuronal viability histology in the systemic ATL therapies. Neither retrograde ATL group followed this trend. Similar tendencies are found between the MAP-2 and functional preservation. The lack of protection of retrograde ATL further supports the notion of divergent, even competing, mechanisms involved with the different timing of ATL delivery. Finally, aside from the combined ATL therapy group, the neuronal viability and MAP-2 appear to demonstrate similar results.

Our laboratory has long speculated that inflammation plays a major role in several organ types of ischemia-reperfusion injury. As shown in studies of lung ischemia reperfusion, spinal cord MPO in this study was significantly lower than IR in all therapies that received sys-

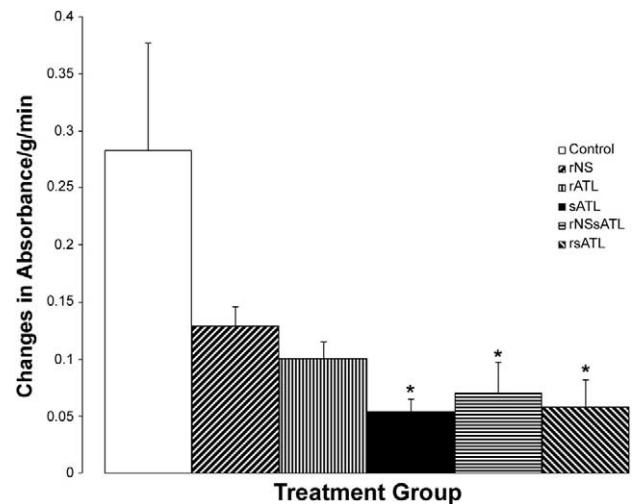


Fig 7. Myeloperoxidase assay results 24 hours after spinal cord ischemia reperfusion. Myeloperoxidase is expressed as mean change in absorbance per milligram per minute  $\pm$  SE. The groups receiving systemic ATL-146e had significantly less neutrophil sequestration than control.\* $p < 0.01$  compared with control, rNS, and rATL. (NS = normal saline; r = retrograde; s = systemic; control = open bar; rATL = vertical striped bar; rNS = right-hatched bar; rNSsATL = horizontal striped bar; rsATL = left-hatched bar; sATL = solid bar.)

temic ATL [14]. Retrograde ATL and retrograde NS alone both had trends toward lower MPO, but may have represented a mechanism of vascular washout of ischemic mediators rather than inflammatory inhibition. In this study, reduced neutrophil sequestration again mirrored functional outcome in all groups except for the combined ATL group. This group worsened as a whole after 24 hours despite lower MPO levels. Again, the mode of attenuation of injury in this group appears to diverge from that of the other groups.

The results of this study imply that the protective properties of ATL-146e are not entirely attributable to the anti-inflammatory properties of the drug and that adenosine A<sub>2A</sub> receptor activation may activate other mechanism in response to injury. Other studies have shown that inflammatory inhibition alone using glucocorticoids attenuated some of the ischemic spinal cord injury, but did not completely prevent it [24]. Therefore, we propose that the protective effects of ATL-146e are multifactorial, not limited to reduction in inflammation. A very likely divergent mechanism of ATL neuroprotection may be vascular dilatation. Protective vasodilatation would be consistent with studies that have used papaverine to prevent vasoconstriction in hypothermic protective strategies [25]. Assuming a multifactorial mechanism of protection, we suspect that inflammatory inhibition still plays a significant role in protection against spinal cord ischemia-reperfusion injury. We plan to continue to better define role of inflammation in this process by evaluating cytokines in the cerebrospinal fluid and tissue inflammatory markers in future studies.

We acknowledge that this study has limitations. First, the reperfusion period of 24 hours is short. But this study and previous examples have shown that significant injury can be both documented and attenuated within this time period. Next, a longer reperfusion period may have been very helpful in further defining the declining course of the combined retrograde and systemic ATL group and for discerning differences among the treatment groups. The detrimental outcome in the combined therapy group may be helpful for future studies of longer-acting adenosine A<sub>2A</sub> receptor agonists in terms of optimal timing of delivery. Finally, the measure of inflammation in this study is crude. The MPO reduction does not prove our hypothesis of an anti-inflammatory mechanism of ATL-146e, but remains a finding that supports this hypothesis. As stated earlier, future studies are intended to expand upon this finding.

In conclusion, retrograde therapy through the hemiazygous vein can deliver local therapy to the spinal cord microvasculature. Furthermore, retrograde delivery may preserve early spinal cord function, but does not lead to significant preservation of cytoarchitecture or reduction in neutrophil sequestration. However, this study demonstrates that systemic therapy alone with the selective adenosine A<sub>2A</sub> agonist ATL-146e preserves spinal cord form and function, presumably by impaired recruitment of systemic inflammatory cells into the injured tissue. Although significant direct differences between retrograde and systemic delivery of ATL-146e are not found in

this study, we conclude that systemic therapy adenosine A<sub>2A</sub> at reperfusion receptor activation should be the preferred mode of delivery because it requires less intervention, it better preserves cytoarchitecture compared with control, and it better limits neutrophil sequestration compared with control. The use of systemic ATL-146e at reperfusion may play a significant role in the prevention of neurologic deficits after surgery of the thoracic aorta in the future.

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## INVITED COMMENTARY

Lower limb neurologic deficits are a devastating complication of thoracoabdominal aortic aneurysm (TAAA) repair, and result from a prolonged or permanent interruption of blood supply to the spinal cord. Protective measures try to preserve spinal cord perfusion (ie, cerebrospinal fluid drainage, reattachment of intercostals arteries, distal aortic perfusion, and maintaining adequate proximal and distal pressures, in combination with monitoring spinal cord function with motor evoked potentials). However, if a period of spinal cord ischemia can not be avoided, it would be advantageous if adjuncts were available that enhance neuronal tolerance and improve neuronal survival after a temporary interruption of blood supply. If blood flow to the spinal cord can not be restored during the procedure, protective measures operate in the small watershed area of the infarction and are probably of small significance in the spinal cord.

The neuroprotective effect of permissive or induced mild hypothermia is well established in the experimental setting, but may be complicated by a deterioration of hemostasis, cardiac arrhythmias, and postoperative infections in the clinical situation.

The role of pharmacologic interventions during TAAA surgery has to be established. At best the tolerable duration of temporary spinal cord ischemia can be increased. The article by Reece and associates [1] clearly describes that the systemic route of administration of the adenosine A<sub>2A</sub> agonist ATL-146e improved functional and immunohistochemical outcomes in a porcine model of temporary spinal cord ischemia. The search for the golden pharmacologic neuroprotective bullet extends several decades. The majority of putative neuroprotective agents are hampered for clinical use by serious side effects. Moreover, in clinical stroke, apparently promising neuroprotective pharmacologic agents were not effective in large clinical studies, although experimental results showed significant improved outcomes. The lack of effectiveness is probably due partly to differences in between animal models and the clinical situation, but it can

also be attributed to flaws in the preclinical studies. Indeed systematic review of experimental studies in stroke revealed serious flaws in the methodology and did not show convincing evidence to substantiate the decision to perform clinical trials. Consequently, guidelines were suggested for animal studies in stroke that should be adapted prior to starting a clinical study of a potential neuroprotective agent. If adjusted for TAAA surgery and experimental spinal cord ischemia a modified version of these guidelines may be of use in experimental spinal cord ischemia:

1. Blinded drug administration and blinded outcome evaluation, which are essential precautions against bias in clinical trials should be valued equally in animal studies.
2. Monitoring physiologic parameters.
3. Group size based on a-priori power calculations.
4. Assessment of both histopathologic and functional outcomes.
5. Outcome assessment in the acute phase (up to 6 days) and in the chronic phase (up to 30 days).

These recommendations may be of use in order to avoid overoptimistic expectations of proposed neuroprotective agents in the clinical application during TAAA surgery.

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