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Early adenosine receptor activation ameliorates spinal cord reperfusion injury

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Abstract

Objectives—Adenosine receptor activation at reperfusion has been shown to ameliorate ischemia—reperfusion injury of the spinal cord, but the effects of therapy given in response to ischemic injury are unknown. We hypothesized that adenosine receptor activation with ATL-146e would produce similar protection from ischemic spinal cord injury, whether given at reperfusion or in a delayed fashion.

Methods—Twenty-two New Zealand white rabbits were divided into three groups. All three groups, including the ischemia—reperfusion group (IR, $n = 8$), underwent 45 min of infrarenal aortic occlusion. The early treatment group (early, $n = 8$) received 0.06 $\mu\text{g/kg/min}$ of ATL-146e for 3 h beginning 10 min prior to reperfusion. The delayed treatment group (delayed, $n = 6$) received ATL-146e starting 1 h after reperfusion. After 48 h, hind limb function was graded using the Tarlov score. Finally, lumbar spinal cord neuronal cytoarchitecture was evaluated.

Results—Hemodynamic parameters were similar among the groups. Hind limb function at 48 h was significantly better in the early group (3.5 ± 1.0) compared to the IR group (0.625 ± 0.5 , $P \leq 0.01$). There was a trend towards better hind limb function in the early group compared to the delayed group (2.4 ± 1.1 , $P = 0.08$). Hind limb function was similar between delayed and IR groups. Hematoxylin—eosin spinal cord sections demonstrated preservation of viable motor neurons in the early group compared to the delayed and IR groups.

Conclusions—Early therapy with ATL-146e provided better protection in this study; therefore, therapy should not be delayed until there is evidence of ischemic neurological deficit. This study suggests that adenosine receptor activation is most effective as a preventive strategy at reperfusion for optimal protection in spinal cord ischemia—reperfusion injury. *J Cardiovasc Med* 9:363–367 © 2008 Italian Federation of Cardiology.

Keywords

adenosine; paraplegia; spinal cord ischemia; vascular surgery

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Introduction

Spinal cord injury resulting from ischemia and reperfusion remains a real and significant risk for patients undergoing procedures involving the thoracic and thoracoabdominal aorta [1-5]. Paraplegia rates can approach 20% in the highest risk groups undergoing aortic reconstruction involving the descending aorta. Several protective strategies for the prevention of paraplegia in these patients are currently advocated. These strategies include various local and regional hypothermia, cerebrospinal fluid drainage, and selective arterial perfusion, all of which can lower the paraplegia risk to less than 10% [6-9]. Most preventive strategies have not included pharmacological agents because the medications used to date have had limited success. Recently, however, adenosine receptor activation at reperfusion has shown promise for attenuating spinal cord ischemia—reperfusion injury. Although recent studies suggest that the effectiveness of preventive ATL-146e may not be limited to adenosine A_{2A} receptor activation, ATL-146e has not been examined as a response to injury in a delayed fashion. Clinically speaking, ATL-146e appears to attenuate injury effectively when given as prophylaxis, but its effectiveness is unknown when delivered in response to injury. This study aimed at evaluating the effectiveness of adenosine A_{2A} receptor activation in response to ischemia—reperfusion injury. We hypothesized that ATL-146e would produce similar protection from ischemic spinal cord injury whether given at reperfusion or in a delayed fashion.

Materials and methods

Procedures

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, DC: National Academy Press; 1996).

The study utilized mature New Zealand white rabbits in a model of normothermic spinal cord ischemia—reperfusion. After induction and endotracheal intubation, animals were ventilated with a volume ventilator at a tidal volume of 20 ml/kg and a rate of 25 breaths/min. Throughout the procedure, animals received vaporized halothane, which was titrated to appropriate sedation. Arterial catheters were placed for blood pressure monitoring, and the marginal ear vein was accessed for delivery of both normal saline and any intravenous therapy.

The animals were divided into three groups: an ischemia—reperfusion group (IR), a preventive ATL treatment group (early), and a delayed ATL treatment group (delayed). Ischemia—reperfusion control animals (IR, $n = 8$) underwent laparotomy with 45 min of ischemia by cross-clamping the infrarenal aorta and inferior vena cava. In addition, the aorta and the inferior vena cava were cross-clamped at the iliac bifurcation to prevent any retrograde perfusion to the spinal cord. IR group animals received an intravenous saline infusion during reperfusion. The final two groups underwent identical procedures to the IR group except that immediate ATL-146e animals (early, $n = 8$) received 0.06 µg/kg/min of ATL-146e for 3 h beginning 10 min prior to reperfusion, and the delayed ATL-146e animals (delayed, $n = 6$) received 0.06 µg/kg/min of ATL-146e for 3 h beginning 1 h after reperfusion. All drugs were given using a Harvard micro-infusion pump. The delivery and dose of ATL-146e in the early group were based on our previous work demonstrating efficacy of treatment beginning just prior to reperfusion. The intention of the treatment was to attenuate reperfusion injury rather than reduce the ischemia.

The animals were recovered until they were able to lift their heads on their own. All animals that were unable to sit up independently received a bolus of intravenous fluids twice daily and

were hand-fed. Owing to concerns for humane treatment, our institution does not allow survival of animals beyond 48 h without hind limb function graded at 3 or better. At 48 h, all animals were again anesthetized if they were unable to sit up on their own. Then they were perfusion fixed with formalin. Spinal cords were harvested and placed in formalin for histology and immunohistochemistry. The remaining animals were followed for 7 days to demonstrate any longer term changes in functional ability over the first week.

Function

The Tarlov scale was used to assess functional outcomes at 12, 24, 36, and 48 h and then daily for up to 7 days. The Tarlov scale grades the animals based on their ability to move, sit, and hop. A score of 0 indicates complete paraplegia with no movement in the hind limbs. A score of 1 indicates that the animal has some hind limb movement. A score of 2 signifies that the animal can sit with assistance, while 3 means it can sit on its own. A score of 4 shows the animal has a weak hop, and 5 means the animal has a normal hop. The reported scores were graded by a blinded observer with no tie to the project.

Neuronal viability

Fixed spinal cord specimens from the region of ischemic injury (lumbar) were sectioned into 5 μ m sections. Serial sections of each spinal cord were stained for hematoxylin—eosin. These sections were graded by a blinded observer by counting the number of viable motor neurons per high powered field. Neurons were considered nonviable based on loss of nuclear structure, cellular retraction from the surrounding tissue, and loss of cellular architecture. Owing to the range of reperfusion times at spinal cord procurement, the comparisons can, however, only be qualitative in nature.

Statistics

Tarlov scores of neurobehavioral function of the three groups were analyzed using analysis of variance (ANOVA) with Bonferroni multiple comparison post-hoc test to determine significant differences. Assumptions were tested and nonparametric test results verified the results of the ANOVA. The statistics for this study were performed by an independent statistician.

Results

Hemodynamic parameters

No differences were identified among groups during ischemia or reperfusion in terms of heart rate, blood pressure, or core body temperature.

Functional outcomes

Tarlov scores at 12, 24, 36, and 48 h are depicted in Fig. 1. At all time points, the early group had significantly better function compared to the IR group (3.5 ± 1.0 vs. 0.625 ± 0.5 , each $P \leq 0.01$). By 48 h, the hind limb function of the early group was approaching, although not reaching, significance (2.4 ± 1.1 , $P = 0.08$). Hind limb function was not significantly different between delayed and control groups ($P \geq 0.2$). Animals surviving 7 days showed no further functional deterioration. The number of animals surviving 7 days (with a Tarlov ≥ 4) was significantly higher in the early group (5/8) compared to both the delayed (2/6) and IR groups (0/8).

Neuronal viability

Light microscopic review of hematoxylin—eosin-stained spinal cord tissue from IR animals revealed extensive dissolution of cytoarchitecture, with vacuolization of grey and white matter and widespread loss of ventral horn neurons. Review of tissue from early ATL-treated animals

demonstrated mild cytoarchitectural derangement with preservation of large numbers of ventral horn neurons, whereas spinal cord tissue from delayed ATL-treated and IR animals revealed moderate degenerative cytoarchitectural changes and multiple pyknotic neuronal somata in the ventral horn. Again these comparisons are purely qualitative because the spinal cords were not all procured at identical time points. Representative hematoxylin—eosin sections of lumbar spinal cords are demonstrated in Fig. 2.

Discussion

Paraplegia remains a devastating complication of thoraco-abdominal aortic reconstruction for a significant number of patients. The resulting strain on families and the medical system can be tremendous in terms of social, medical, and financial burdens. Prevention of paraplegia in patients undergoing thoracic aortic reconstruction has been attempted using strategies to preserve spinal cord perfusion through perfusion of collateral vascular beds, limiting neuronal metabolism through spinal cord cooling, or reducing spinal compartment pressures through lumbar fluid drainage. Although these neuro-protective strategies have been shown to decrease rates of neurological injury, a real risk of paraplegia remains for patients undergoing thoracic and thoracoabdominal vascular procedures. Furthermore, all of these approaches require extra procedures, which both take more time and have some potential risks of their own.

In theory, a pharmacological protective strategy could avoid the additional complex procedures and time, as it could be delivered through peripheral intravenous access. Previously, we have had success with delivery of an adenosine agonist at reperfusion in an animal model of spinal cord ischemia—reperfusion [10-12]. The results of the present study support the conclusions of our previous investigations demonstrating that adenosine receptor activation at reperfusion of an ischemic spinal cord insult can attenuate functional injury. In this study, ATL treatment beginning 10 min prior to reperfusion significantly preserved hind limb function. Additionally, there was a qualitative preservation of spinal cytoarchitecture using this strategy, as shown previously [13,14]. ATL given at or just prior to reperfusion effectively attenuates injury in animal models of spinal cord ischemia—reperfusion.

More importantly, this study also intended to evaluate a delayed delivery of ATL-146e. If this approach proved successful, therapy could potentially be tailored to those patients demonstrating neurological deficits in the postoperative period. Unfortunately, delayed therapy did not produce results as promising as therapy at reperfusion, suggesting that adenosine receptor activation is a better preventive than rescue strategy. Early delivery of ATL at reperfusion appeared to have some benefit compared with delayed therapy in terms of functional outcomes after 48 h of reperfusion, but lacked statistical significance. Significantly more animals, however, maintained near-normal Tarlov scores over time when treated early rather than late. Moreover, the qualitative cytoarchitectural outcomes were worse in the delayed therapy groups. These findings imply that delayed adenosine A_{2A} receptor activation is not the optimal time for spinal cord protection from ischemia—reperfusion injury.

We found previously that neurological injury is present prior to markers of injury, including tumor necrosis factor release or cytoarchitectural changes [15]. Early markers of spinal cord injury from ischemia and reperfusion have not been identified to date [16]. Early identification of injury may limit the need for preventive therapy, but our present ability to identify injury occurs after we could have potentially attenuated the injury. Similar difficulties have been demonstrated in the controversial treatment of traumatic spinal cord injury. Although the merits of steroid therapy in blunt spinal cord injury can be debated, it is clear that delaying therapy beyond the first few hours is not effective [17,18]. The ineffectiveness of delayed therapies in trauma and in cardiovascular surgery is thought to be due to early inflammation, which exacerbates local injury through activation of neutrophils and influx of fluid into the spinal

cord parenchyma [19-21]. The final result is increased cytotoxic mediators leading to further secondary injury and increased interstitial pressures limiting spinal cord perfusion. Further argument for early therapy is that adenosine A_{2A} receptor activation may attenuate cellular metabolism. Decreasing neuronal metabolism could limit excitotoxicity and the eventual neurological injury [15,22-26]. No matter what the mechanism, this study suggests that adenosine A_{2A} activation with ATL-146e should be administered at reperfusion rather than after reperfusion to maximize the protective benefits to the spinal cord.

The current study does have limitations. The study period ranged from 48 h to 7 days. This design was intended to show the sustainability of neuroprotection from adenosine A_{2A} receptor activation. Unfortunately, this range limited direct comparison of the cytoarchitectural outcomes to a qualitative comparison. This design, however, allowed us to show that the animals with preserved function did not deteriorate over the week following reperfusion. Additionally, the study was only able to demonstrate a trend towards better preservation of function by immediate therapy compared to delayed therapy. However, immediate therapy clearly improved outcomes compared to ischemic controls, while delayed therapy showed no significant difference compared to ischemic controls. The delayed therapy functional results approached ischemic controls but with a higher standard error. The effects of delayed therapy were more erratic with the average animal regaining only minimal hind limb movement. Despite these limitations, we feel this study shows that immediate treatment of ischemic spinal cord injury is effective and delayed therapy is suboptimal.

In conclusion, delayed delivery of ATL-146e does not appear to ameliorate ischemia—reperfusion injury of the spinal cord as well as therapy delivered just prior to reperfusion. Therefore, adenosine receptor activation should not be delayed until there is evidence of ischemic neurological deficit. This study suggests that adenosine agonist therapy should be employed prophylactically in all at-risk patients, with drug administration at reperfusion, for optimal neurological protection in spinal cord ischemia—reperfusion from temporary aortic occlusion.

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Fig. 1.

Hind limb function. Hind limb function is defined by the Tarlov score over the first 48 h. Of note, the early group demonstrates significantly better function over the ischemia—reperfusion group (IR) at every time point. The benefit of the early group approaches significance compared to the delayed group ($P = 0.08$).

Fig. 2.

Lumbar spine cytoarchitecture. These hematoxylin—eosin sections of the lumbar spinal cord demonstrate the qualitative differences in cytoarchitecture among the groups. The early group on the left is most preserved showing mild cytoarchitectural derangement from normal grey and white matter cytoarchitecture, with large pyramidal ventral horn motor nuclei and prominent nucleoli. The delayed and ischemia—reperfusion groups represented on the right contain markedly abnormal spinal cord sections with vacuolization of parenchyma and multiple pyknotic neuronal somata. Arrows point at nuclei in both sections.