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Adenosine A_{2A} Analogue Reduces Long-Term Neurologic Injury after Blunt Spinal Trauma[1,2]

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Background. **ATL-146e is an adenosine A_{2A} agonist that has recently been demonstrated to improve neurological outcome in spinal cord injury in animals. In the current study, we extended the treatment paradigm and tested neurobehavioral functioning out to 1 week after injury to assess if early neurological improvement is sustained long term by an adenosine analogue.**

Materials and methods. **New Zealand White rabbits (3.0–3.5 kg) sustained mid-thoracic blunt spinal cord injury using a weight-drop model (10 g weight dropped from 6 cm directly onto dura). Animals received either (1) 3 h iv infusion of saline carrier (Trauma, $N = 21$); (2) 3 h iv infusion of 0.06 μg/kg/min ATL-146e followed by intraperitoneal bolus of 10.8 μg/kg ATL-146e at 3 h postinjury (ATL, $N = 14$); or (3) 3 h iv infusion of 0.06 μg/kg/min ATL-146e followed by intraperitoneal bolus injection of 10.8 μg/kg ATL-146e at 3, 12, and 24 h postinjury (ATL-PLUS, $N = 11$). Fourteen animals underwent sham injury. Hemodynamic parameters were monitored and hind limb motor functioning was assessed by Tarlov scores (0 = paralyzed to 5 = normal hop) for 7 days after injury.**

Results. **ATL-146e significantly improved Tarlov scores of ATL-146e groups compared with saline-treated controls ($P < 0.01$ 12, 24, 36, and 48 h). Control animals, severely neurologically impaired at 48 h (Tarlov 1.61 ± 0.35), were euthanized early due to ethical concerns, thus not permitting later statistical comparisons. Early neurological improvements in both ATL-146e-treated groups were sustained longer term (7 day mean Tarlov, SHAM 4.9 ± 0.30, ATL 5.0 ± 0, ATL-PLUS 4.25 ± 0.31).**

Conclusions. **ATL-146e given immediately after blunt spinal cord trauma significantly improves neurological outcome, which is sustained through 7 days. Early adenosine A2A receptor agonism may be critical since additional IP administration afforded no further neurological improvement. The current data further support the potential clinical utility of adenosine A_{2A} agonists in the treatment of spinal cord injury.**

Key Words**: spinal cord injury; trauma; adenosine; A_{2A} receptor; inflammation; apoptosis; motor function; hemodynamics; rabbit.**

INTRODUCTION

Approximately 14,000 cases of spinal cord injury occur each year in the United States. Spinal cord injury is most common between the ages of 20 and 40 and patients face life-long morbidity from paralysis, pain, and loss of sexual and bowel/bladder function [1]. The annual societal cost makes spinal cord injury a paramount healthcare issue in our society.

The current standard of care for spinal cord injury involves early administration of high-dose intravenous steroids. Steroid therapy is thought to improve outcome via its anti-inflammatory properties as well as its antioxidant properties. Multi-center prospective clinical trials revealed a modest benefit from methylprednisolone therapy; however, the consequences of these trials have been controversial due to questions about study design and data analysis created debate [2–4]. Later studies have produced conflicting results and the

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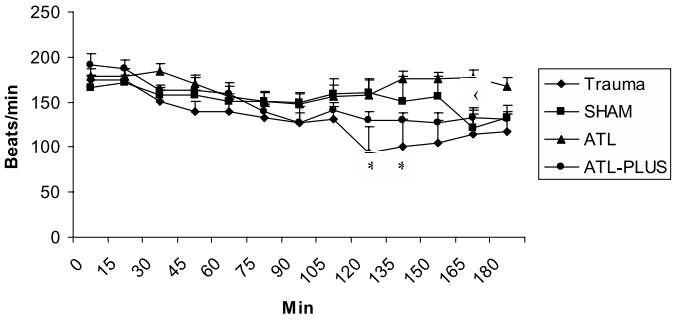

FIG. 1. Mean postoperative heart rates. Although a few isolated time points show significant differences, there are no consistent differences. *$P < 0.05$ Trauma *versus* ATL, †$P < 0.05$ ATL *versus* SHAM.

utility of corticosteroids in spinal cord injury remains controversial [5, 6]. Furthermore, the efficacy of high-dose steroid therapy to produce sustained improvements in spinal cord functioning has been questioned in addition to concerns about adverse side effects [7].

As high-dose steroid therapy for this injury receives increasing scrutiny, the search for alternative therapies has intensified. Adenosine receptor analogues are a promising group of candidate compounds. Adenosine A_{2A} receptors modulate several pathophysiologic mechanisms that are known to occur in spinal cord injury [8]. Leukocyte infiltration and production of tumor necrosis factor alpha (TNF-α) are markedly increased following nervous system trauma. Adenosine A_{2A} receptor activation suppresses neutrophil recruitment and reduces TNF-α release by monocytes [9]. Adenosine A_{2A} receptor agonism may also provide protection to the injured spinal cord by its vasodilatory effects on neural microvasculature and possibly by decreasing the metabolic rate of neurons [10].

In a previous study from our laboratory, Cassada et al. demonstrated that ATL-146e, a selective adenosine A_{2A} receptor agonist, improved neurological function in the first 12 h following blunt spinal cord trauma in the rabbit [11]. The current study was designed to determine if this functional protection persists following blunt spinal cord trauma. We assess whether treatment with ATL-146e results in neurological improvement sustained to 7 days in rabbit spinal cord trauma. Additionally, we investigate whether additional intraperitoneal (IP) doses of ATL-146e administered at multiple postinjury time points would further improve neurological outcome.

MATERIALS AND METHODS

All protocols were approved by the Animal Care and Use Committee of the University of Virginia. All animals received humane care according to the Guide for the Use of Laboratory Animals.

Induction of Spinal Cord Injury

New Zealand White rabbits (3.0–3.5 kg) underwent blunt spinal cord trauma in a manner previously described [11]. Briefly, rabbits were anesthetized with ketamine and xylazine before endotracheal intubation. Anesthesia was maintained with inhaled halothane titrated to effect. Arterial catheters were placed in the ears for blood pressure monitoring, and the marginal ear vein was accessed for delivery of both normal saline and any intravenous therapies. The animals were placed on a heating blanket in a prone position.

The animals were divided into four groups including traumatic controls, short ATL treatment (ATL), long ATL treatment (ATL-Plus), and sham procedure. Sham animals ($n = 14$) underwent a midline incision before laminectomy of the lower thoracic spine was preformed. The dura mater was left intact. The incision was closed in two layers before the animals were recovered. Traumatic control animals (Trauma, $n = 21$) underwent identical exposure of the dura. To create the injury, a 10 g weight was dropped from 6 cm on to a brass impounder rested on the dura to create a controlled, reproducible spinal cord injury. The other two groups were subject to identical injury. ATL-146e (ATL, $n = 14$) animals received 0.06 μg/kg/min IV of ATL-146e for 3 h beginning 10 minutes after reperfusion with an IP bolus of ATL-146e (10.8 μg/kg) after the completion of the IV therapy. The second treatment group (ATL-PLUS, $n = 11$) received the same therapy (0.06 μg/kg/min IV ATL-146e for 3 h beginning 10 min after the injury) and were then treated with additional IP boluses of ATL-146e (10.8 μk/kg) after completion of the 3-h infusion and again at 12 and 24 h postinjury. All anterior spinal elements remained intact to preserve structural stability and prevent further injury.

The animals were recovered until they were able to lift their heads. Animals were survived for 48 h while getting baytril and buprenex every 12 h. All animals that were unable to sit up independently received a bolus of intravenous fluids twice per day plus they were hand fed. At 48 h, all animals unable to sit without assistance were euthanized for ethical reasons. All animals able to sit under their own power were survived for 7 days.

Hemodynamic Monitoring

During the procedure and postoperatively, arterial blood pressures were noted every 15 min and recorded for evaluation of procedural and pharmacological effects on the animals. Arterial blood pressures were later examined by ANOVA and the Bonferroni multiple comparison test to assess statistical differences.

Hind Limb Motor Function Assessment

The Tarlov Scale was used to assess hind limb motor functional outcomes at 12, 24, 36, and 48 h and then daily thereafter in all

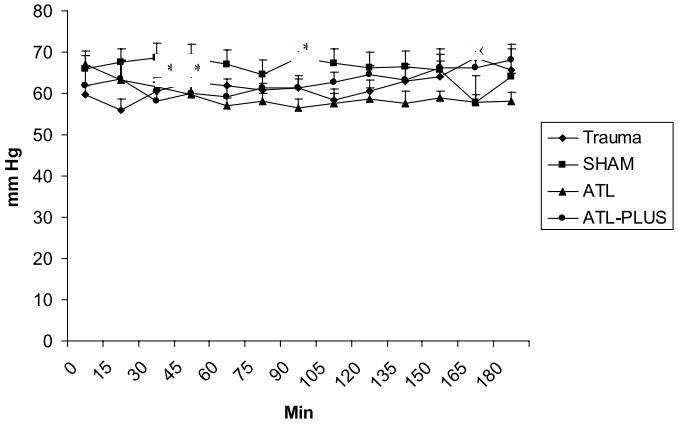

FIG. 2. Mean postoperative mean arterial pressures. Again few isolated time points have significantly different mean arterial pressures, but the differences are not consistent. *$P < 0.05$ SHAM *versus* ATL, †$P < 0.05$ ATL *versus* Trauma.

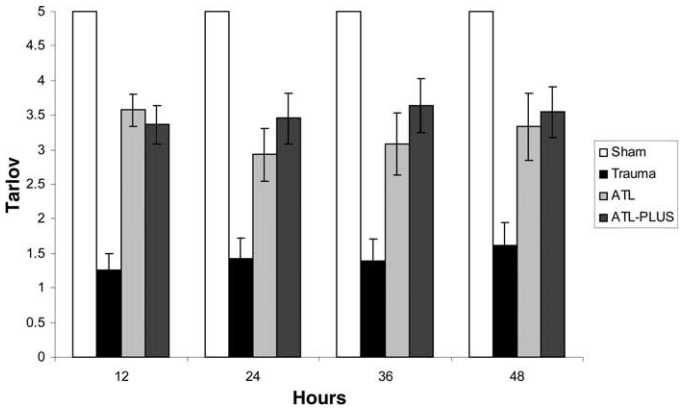

FIG. 3. Mean Tarlov scores during first 48 h. No statistical differences between the sham and treatment groups can be identified, but both the treatment groups and the sham group have significantly better function than the trauma group.

animals who could sit without assistance. The Tarlov scale is a well-characterized, reliable method of grading hind limb motor function. Tarlov scores are graded on a scale of 0 to 5: 0, atony; 1, slight movement; 2, sits with assistance; 3, sits alone; 4, weak hop; 5, normal hop. For ethical reasons, animals with Tarlov scores no greater than 2 were euthanized at 48 h. Tarlov scores were performed daily by a blinded member of the veterinary staff with no tie to the project. All functional data were compared using Kruskal–Wallis Nonparametric tests and χ^2 Analysis.

RESULTS

Hemodynamic Parameters

Postoperative hemodynamic variables did not differ significantly among the four groups (Fig. 1). Similarly, there were no significant differences in mean arterial pressure demonstrated no significant differences among groups (Fig. 2).

Neurological Outcome

Neurological outcomes were significantly preserved by the use of ATL-146e without and with extended IP bolus dosing. At 12 h postinjury, Tarlov scores were significantly higher in ATL and ATL-PLUS compared with control (ATL 3.6 ± 0.23 and ATL-PLUS 3.4 ± 0.28 *versus* Trauma 1.3 ± 1.0, $P < 0.01$). Sham animals were significantly better than all groups over the first 48 h (5 ± 0, $P < 0.01$). We found similar results for function at 24, 36, and 48 h (Fig. 3, ATL, ATL-PLUS and SHAM *versus* Trauma, $P < 0.01$). After 2 days, animals with Tarlov scores ≤ 2 were euthanized for ethical reasons (six Trauma animals after 48 h); animals able to sit under their own power were survived. Function permitting survival through 7 days in the remaining animals (Tarlov score ≥ 3) was possible in 1 of 8 (12.5%) of control animals, 100% of sham animals, 10 of 14 (71%) of ATL animals and 8 of 11 (73%) of ATL-PLUS. Function permitting survival was significantly increased in both treatment groups and sham compared to trauma controls ($P < 0.01$). In addition, hind limb motor functioning did not deteriorate in animals with function permitting survival (Tarlov scores ≥ 3) over postinjury days 5 to 7 (Fig. 4).

DISCUSSION

The current study confirms our initial results showing that ATL-146e therapy administered early after spinal cord injury improves neurological outcome. This study found sustained preservation in hind limb motor function between ATL-146e-treated and control groups through 7 days postinjury. The sustained preservation of function with ATL therapy suggests the assertion that this compound may be useful for treating spinal cord injury.

The standard of care for patients with spinal cord injury today remains steroid therapy, usually with methylprednisolone. The use of steroids is based on the series of National Acute Spinal Cord Injury Studies (NASCIS I, II, and III) [2–4]. The NASCIS trials were randomized, prospective clinical trials that demonstrated short-term improvement in functional outcome with high-dose intravenous methylprednisolone. NASCIS III also demonstrated the potential adverse effects of steroid therapy as extended treatment was associated with higher rates of sepsis and pneumonia [3]. Several other studies have questioned the methylprednisolone data, showing no improvement of steroid therapy over control [5, 12, 13]. Additionally, immune response alterations, higher rates of pneumonia, and longer hospital stays were noted in steroid-treated patients [7]. It is thus disappointing that steroids remain the standard of care for the treatment of spinal cord injury, even though they were first used 20 years ago and their utility remains in dispute in the spinal cord injury literature. The use of a selective adenosine A_{2A}

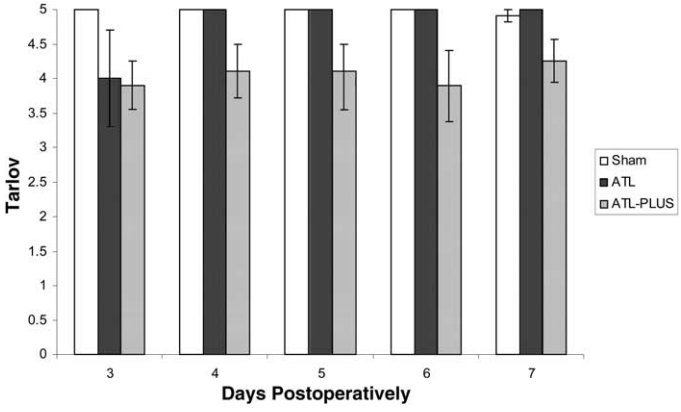

FIG. 4. Mean Tarlov scores of animals able to survive longer than 48 h. There are no differences between the surviving animals in these groups, but the poorest functioning animals are no longer included in this time point. However, the mean functional scores are clearly sustained in days 3 through 7.

receptor analogue for blunt spinal cord injury may have several potential advantages over steroids. While the mechanism of action of methylprednisolone is thought to be as an anti-inflammatory and antioxidant, many actions of steroids require activation of transcriptional events that may take too long to manifest. The adenosine A_{2A} receptor is a Gs-protein coupled receptor that increases cAMP levels and exerts anti-inflammatory effects in leukocytes and lymphocytes within seconds [14]. Adenosine A_{2A} receptor activation leads to inactivation of neutrophils and reduces cytokine release, most notably TNF-α by monocytes [9, 15–17]. Importantly, ATL-146e does not appear to be immunosuppressive. The extent of immune modulation has not been proven to date, but the animal models have not shown any evidence of increased incidence of infection.

ATL-146e also has non-inflammatory/immunological properties that may contribute to its observed neuroprotective effects. Hypoxic-ischemic injury occurs within the spinal cord and develops early after mechanical injury due to disruption in blood flow. This is particularly damaging to gray matter due to its high metabolic rate. The local microvasculature has A_{2A} receptors, activation of which causes vasodilatation [18, 19]. By this route adenosine A_{2A} receptor activation may increase local blood flow and limit hypoxic-ischemic damage in spinal cord injury. The combination of upstream anti-inflammatory mechanism and lack of over-exuberant immune suppression with possible increase in local postinjury perfusion indicates that ATL may be a more than promising alternative to steroids for the treatment of blunt spinal cord trauma.

A possible contraindication to the use of adenosine analogues to treat spinal cord injury is drug-induced hypotension. Hypotension is associated with worsened morbidity and mortality following spinal cord injury. High doses of adenosine or ATL-146e can cause hemodynamic compromise. The dose of ATL-146e used herein (0.06 μg/kg/min for 3 h) did not adversely affect mean arterial pressure or heart rate. This observation is consistent with previous work with ATL-146e at this dose [20]. Furthermore, no toxicity has been noted at this dose in previous studies using ATL-146e.

The current study demonstrates that ATL-146e preserves spinal cord protection following blunt spinal cord trauma. This effect is sustained for at least 7 days following injury. We found no additional benefit to ATL-146e therapy beyond the initial 3 h treatment, which suggests necessity of early inflammatory blockade following the traumatic injury. ATL-146e, via agonism of the adenosine A_{2A} receptor, is effective in improving outcome from spinal cord trauma and may represent a superior alternative to the steroids for the treatment of patients with spinal cord injury. Further study is required to elucidate the mechanism of protection from ATL-146e therapy in addition to directly comparing the results of ATL-146e with methylprednisolone following blunt spinal cord injury.

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