INTRODUCTION: Spinal cord trauma with paralysis is a devastating cause of long-term suffering and disability. Following primary trauma, the subsequent inflammatory response generates a secondary injury. Leukocyte adherence to and extravasation through the endothelium of capillaries and postcapillary venules adds insult through the release of inflammatory mediators into the zone of injury. This secondary inflammatory response damages the endothelium and induces microcirculatory disturbances leading to further spinal cord ischemia. Current acute treatment for spinal cord injury involves the administration of intravenous methylprednisolone (MP). MP’s anti-inflammatory effects require induction of gene transcription with subsequent delayed onset of action. The efficacy of MP in a rat spinal cord injury model has recently been questioned(1).

Adenosine is an important neuromodulator in the central nervous system. Adenosine is a non-specific agonist that accumulates in and is released from ischemic and hypoxic tissues. The A₂a receptor is G-protein coupled to the cyclic adenosine monophosphate second messenger pathway with corresponding immediate effects on intracellular free calcium. Adenosine has been found to inhibit leukocyte activation and endothelial adherence as well as the release of inflammatory mediators. The adenosine analogue ATL202 (Adenosine Therapeutics, VA) was designed for A₂a receptor specificity, increased half-life, and potent inhibition of inflammatory cells. Adenosine A₂a analogues have been reported to improve neurologic outcome in a rabbit spinal cord injury model(2).

We hypothesize that the treatment of traumatic spinal cord injury with an adenosine A₂a analogue (ATL202) decreases locomotive deficit via the downregulation of the host inflammatory response and resultant decrease in secondary injury in a rat model.

METHODS: Sprague-Dawley female rats were divided into three treatment groups. A control group received a normal saline (NS) bolus, an adenosine analogue bolus group received a 600ng bolus of ATL202, and an adenosine analogue bolus and pump group received a 600ng bolus and an osmotic pump (Alzet, CA) infusing 60ng/hour for 72 hours of ATL202. A preliminary study was undertaken to determine the appropriate dose of ATL202 for bolus and
infusion in the rat model employed. Furthermore, a pilot group received both the bolus and pump of adenosine analogue and MP (60mg/kg bolus with an infusion of 5.4 mg/kg/hour of methylprednisolone sodium succinate). The rats were anesthetized and a three level mid-thoracic laminectomy was performed. A reproducible traumatic spinal cord injury was inflicted on the rats with the Infinite Horizons Spinal Cord Impactor (Precision Systems and Instrumentation, KY) with an impulse of 150 kilodynes and a velocity of 50 mm/sec. Fascia and skin were close in two layers and all rats received a 5mL intraperitoneal bolus of warm NS and treatment one minute following injury. Locomotor scoring with the BBB Scale(3) was undertaken at 2, 7, and 14 days. The rats were euthanized at 14 days and the injured spinal cord segment harvested for histologic evaluation. An ANOVA with post hoc analysis was employed to compare BBB scores between treatment groups and time points. Statistical significance was defined with a p-value<0.05 and a trend in the data was defined with a p-value<0.15. All animal experimental protocols were approved by the Animal Care and Use Committee.

RESULTS: The average BBB locomotor score ± standard deviation is presented in Figure 1 for the control group (n=7), adenosine analogue bolus group (n=6), and adenosine analogue bolus and pump group (n=11). Statistical significance was demonstrated within and between several groups. Statistically significant improvement in the BBB locomotor scores at the 14 day time point relative to the 2 day time point was demonstrated within all groups (p<0.05). The data between groups demonstrated that the adenosine analogue bolus and the adenosine analogue bolus and pump treatment groups had statistically significant increases in BBB locomotor scores relative to the control at the 2 day time point (p=0.05). Furthermore, the pilot group of adenosine analogue and MP bolus and pump (n=4) demonstrated a trend with an improvement at the 2 day time point relative to control (p=0.11).

CONCLUSION: This study investigated the protective effects of a novel adenosine2A analogue following traumatic spinal cord injury in a rat model. In general, the BBB locomotor scores improved over the 2 week time frame within all groups. The data demonstrated that the adenosine analogue bolus and the adenosine analogue bolus and pump groups had statistically significant increases in BBB locomotor scores relative to the control group at the 2 day time point (p=0.05) but this difference did not remain significant at the 7 day and 14 day time points. Furthermore, there was a trend towards improvement in the adenosine analogue and MP bolus and pump group at the 2 day time point. In the acute treatment of traumatic spinal cord injury combining a kinetically quick treatment (adenosine analogue) with longer lasting but kinetically slow treatment (methylprednisolone) that have different mechanisms of action offer an attractive synergistic protocol. Future efforts will include collecting more
data to achieve stronger statistical significance and to further explore the combination of adenosine analogue and MP in this rat model. Additional objective outcome measures involving volume of cord injury and immunohistochemical analysis are also underway.

Figure 1: Protective Effects of an Adenosine Analogue Following Traumatic Spinal Cord Injury in a Rat Model

REFERENCES:

If noted, the author indicates something of value received. The codes are identified as: a- research or institutional support; b- miscellaneous funding; c- royalties; d- stock options; e- consultant or employee; n- no conflicts disclosed, and * disclosure not available at the time of printing. For full information, refer to inside of back cover.

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