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Comparison of effects of methylprednisolone and anti-CD11d antibody treatments on autonomic dysreflexia after spinal cord injury

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Abstract

Autonomic dysreflexia is a condition of episodic hypertension that develops after spinal cord injury (SCI). We previously showed that a two-day anti-inflammatory treatment with an anti-CD11d integrin monoclonal antibody (mAb), soon after SCI in rats, reduced the magnitude of dysreflexia for at least 6 weeks. Effects of methylprednisolone (MP), a commonly used neuroprotective treatment for SCI, on dysreflexia have never been examined. We compared the effects of a 2-day MP treatment and/or the anti-CD11d mAb on autonomic dysreflexia, elicited by colon distension, after clip-compression SCI at the 4th thoracic segment (T4) in rats. We assessed the effects of each treatment on the size of the calcitonin gene-related peptide (CGRP)-immunoreactive afferent arbour in the dorsal horn, as changes in this arbour can correlate with the development of dysreflexia. MP reduced autonomic dysreflexia by ~50% at 2 weeks after SCI, but this effect was lost by 6 weeks. At 2 weeks, the combined effects of MP and the mAb were not additive, reducing dysreflexia by ~50%. Neither MP nor the mAb treatment altered the area of CGRP-immunoreactive fibres in the lumbar cord, the crucial input region for dysreflexia initiated by colon distension. However, both treatments led to increased fibre areas in the T9 segment, correlated with greater tissue integrity and smaller lesions, delineated by inflammatory cells. In summary, MP only temporarily decreases autonomic dysreflexia after SCI. The early beneficial effects of both treatments on dysreflexia do not relate to changes in the CGRP-immunoreactive afferent arbour but may correlate with decreased lesion progression.

Keywords: Integrin; Methylprednisolone; Autonomic; Arterial pressure; Afferent arbour; Spinal injury; Inflammation

Introduction

Spinal cord injury (SCI) often results in autonomic dysreflexia, a condition characterized by paroxysmal increases in blood pressure that may lead to severe headaches, strokes and even death. Dysreflexia is more prominent after injuries at or above the midthoracic spinal cord, occurring in 50–90% of people with tetraplegia or high paraplegia (Karlsson, 1999). The development of autonomic dysreflexia has been attributed to changes in the peripheral vascular system and to reorganization of intraspinal reflexes that occur after SCI (Collins and DiCarlo, 2002; Krassioukov et al., 2002; Krenz et al., 1999; Landrum et al., 2002; Llewellyn-Smith and Weaver, 2001; Tang et al., 2003; Weaver et al., 1997). These and other studies suggest that the regulation of blood pressure after SCI depends, to a large extent, on the amount of supraspinal regulation from spared descending pathways (Gris et al., 2004; Mizushima et al., 2003; Weaver et al., 2001). In the absence of a tonic influence from the medulla, the sympathetic spinal circuitry undergoes rearrangements that result in an exaggerated response to sensory stimulation (Krenz and Weaver, 1998; Llewellyn-Smith and Weaver, 2001; Weaver et al., 1997, 2001). These reflexes are triggered by ordinary events, such as a full urinary bladder, distension of the bowel or skin irritation and cause massive increases in arterial pressure (Blackmer, 2004).

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One mechanism underlying alterations in this sympathetic reflex is a nerve growth factor-dependent growth of calcitonin gene related peptide (CGRP)-containing sensory neuron processes into the spinal cord (Krenz et al., 1999; Marsh et al., 2002; Weaver et al., 2001). These studies showed that the magnitude of autonomic dysreflexia correlated with the area of CGRP-immunoreactive fibres in the dorsal horn of the spinal cord. Increases in arterial pressure caused by colon distension are mediated by afferent input to the lumbosacral spinal cord (Vizzard et al., 2000) and changes in the central arbour of those sensory neurons correlate with the magnitude of the reflex responses (Cameron et al., 2003; Krenz et al., 1999).

Among the strategies to treat SCI, interventions designed to diminish secondary tissue damage are viewed as the most promising and feasible (Amar and Levy, 1999; Tator, 1998). Indeed, the plethora of pathologic reactions that result in the enlargement of the lesion after SCI presents an opportunity for therapeutic intervention. Modulation of the early inflammatory response to SCI is an obvious strategy. The broad-spectrum immunosuppressive drug methylprednisolone (MP) is one of the few therapies routinely used after SCI (Fehlings, 2001). However, the neurological outcome after MP treatment is modest and suggests that more selective interventions are required to achieve significant improvement (Bracken, 2001; Fehlings, 2001; Hurlbert, 2000). One such treatment is a 2-day anti-CD11d monoclonal antibody (mAb) treatment that transiently blocks intraspinal infiltration of neutrophils and monocyte/macrophages during the first 3 days after SCI, significantly improving functional recovery, including locomotor and autonomic function and development of pain (Gris et al., 2004). The likely mechanism for this effect is very selective. The initial damage to the endothelial cells of the blood brain barrier results in the release of chemokines and cytokines that trigger the expression of the vascular adhesion molecule-1 (VCAM-1) on the surface of the endothelial cells and the CD11d integrin on the surface of the leukocytes (Grayson et al., 1998; Van der Vieren et al., 1999). The interaction of these molecules allows adhesion and extravasation of the inflammatory cells through the blood spinal cord barrier into the central nervous system.

Despite the significant burden that autonomic dysreflexia brings to everyday quality of life after SCI, a general evaluation of the autonomic function is not usually included in assessment of neurological function when assessing treatments. For example, the effects of MP on autonomic dysreflexia have never been examined. In this study, we evaluated the development of autonomic dysreflexia after an anti-inflammatory treatment with MP and compared these results to the effects of an anti-CD11d mAb treatment. In parallel, we studied one potential mechanism for improvement of autonomic dysreflexia after treatment by the anti-CD11d mAb treatment and MP, namely, changes in the central arbour of CGRP-immunoreactive afferent fibres in the spinal cord dorsal horn. We also included in our study the combination of these treatments as we have shown that this combination can be additive in blocking intraspinal monocyte/macrophage invasion after SCI (Dekaban et al., 2002). We hypothesized that the neurological outcomes of SCI after the combined treatment might be better than after either treatment alone.

Materials and methods

Preparation of animals and treatments

All protocols and procedures were approved by the University of Western Ontario Animal Care Committee in accordance with the policies established in the Canadian Guide to Care and Use of Experimental Animals. Fifty-eight Wistar female rats (Harlan Bioproducts, Indianapolis, Indiana) weighing ~230 g were used in this blinded study. In all rats, the 4th thoracic (T4) segment of the spinal cord was injured by severe compression with a 50-g calibrated clip. Briefly, animals were pre-medicated with diazepam (3.5 mg/kg, i.p., Sabex International Ltd.) and atropine (0.05 mg/kg, s.c., Sigma Chemical) before anesthesia with 1.5–4% halothane and the T4 spinal cord segment was exposed via laminectomy. The modified aneurysm clip (Toronto Western Research Institute, University of Toronto) was applied extradurally around the cord and closed for 1 min. The rats received postoperative care as described previously (Gris et al., 2004).

In a 2-week study, one of the following four treatments was administered in three bolus doses at 2, 24 and 48 h after SCI via tail vein injection. The control group received saline or isotype-matched mAb (1B7) against an irrelevant antigen ($n = 14$), a second group ($n = 7$) received MP (30 mg/kg at 2 h, and 15 mg/kg at 24 and 48 h; Solu-Medrol, Upjohn, Peapack, NJ, USA), a third group ($n = 10$) received the anti-CD11d mAb (1.0 mg/kg) and a fourth group ($n = 7$) received the combination of MP and anti-CD11d mAb simultaneously (doses as above). In a 6-week study of autonomic dysreflexia, one group received MP ($n = 6$), as described above, one group received the anti-CD11d mAb as above ($n = 7$) and the control group ($n = 7$) received the mAb (1B7). The anti-CD11d and 1B7 mAb were generously supplied by the ICOS Corporation (Bothwell, WA).

Assessment of autonomic dysreflexia

Blood pressure and heart rate measurements during autonomic dysreflexia were obtained as described previously (Weaver et al., 2001). In brief, 13 or 40 days after SCI, femoral or carotid arteries, respectively, were cannulated under halothane anesthesia. Six to 8 h later, blood pressure and heart rate responses to balloon (2.0 ml) distension of the colon were tested as a measure of autonomic dysreflexia. Two trials of stimulation were evaluated, separated by a 30-min interval. The testing was repeated the next day and the