Neuroscience 154 (2008) 1627–1638

NEURAL AND ANATOMICAL ABNORMALITIES OF THE GASTROINTESTINAL SYSTEM RESULTING FROM CONTUSION SPINAL CORD INJURY

S. KABATAS,[a,b1] D. YU,[a,b1] X. D. HE,[d] H. S. THATTE,[e] D. BENEDICT,[a,b] K. T. HEPGUL,[f] P. M. BLACK,[a] S. SABHARWAL[b,c] AND Y. D. TENG[a,b,c]*

[a]*Department of Neurosurgery, Harvard Medical School/Brigham and Women's Hospital/Children's Hospital Boston, 75 Francis Street, Peter Bent Building CA 138, Boston, MA 02115, USA*

[b]*Division of Spinal Cord Injury Research, Veterans Affairs Boston Healthcare System, Boston, MA 02132, USA*

[c]*Department of Physical Medicine and Rehabilitation, Harvard Medical School, Spaulding Rehabilitation Hospital, Boston, MA 02114, USA*

[d]*Center for Swallowing and Motility Disorder, Harvard Medical School, Veterans Affairs Boston Healthcare System, Boston, MA 02132, USA*

[e]*Department of Surgery, Harvard Medical School, and Veterans Affairs Boston Healthcare System, Boston, MA 02132, USA*

[f]*Department of Neurosurgery, Istanbul University, Istanbul School of Medicine, Istanbul, Turkey*

Abstract—Gastrointestinal (GI) abnormalities resulting from spinal cord injury (SCI) are challenging disorders that have not been examined experimentally using clinically relevant models. In this study, female Sprague–Dawley rats (n=5/group×4: T10–T11 contusion, laminectomy, or naïve) were fasted for 24 h before being submitted to dye recovery assays (Phenol Red solution, 1.5 ml/rat; per oral) on GI emptying/transiting at 48 h or 4 weeks postinjury (p.i.). Compared with controls, SCI significantly increased dye recovery rate (DRR, determined by spectrophotometry) in the duodenum (+84.6%) and stomach (+32.6%), but decreased it in the jejunum (−64.1% and −49.5%) and ileum (−73.6% and −70.1%) at 48 h and 4 weeks p.i., respectively ($P \leq 0.005$, ANOVA with post hoc t-test). Electrophysiological analysis revealed that purinergic fast inhibitory junction potential (IJP) was reduced ~30% in the antrum and duodenum of rats 48 h p.i. (numbers of animals/numbers of tissue samples=3/7; $P<0.001$), and slow IJP was essentially abolished. Immunocytochemistry consequently uncovered significant reductions in the GI vasoactive intestinal polypeptide and neuronal nitric oxide synthase (i.e. slow IJP mediators) reactivity at 48 h and 4 weeks p.i., suggesting that SCI disrupted interstitial neurotransmission. Importantly, SCI caused discernible atrophy of the GI mucosa and muscle coat (e.g. the two layers of gastric wall were correspondingly 28% and 27% thinner 4 weeks p.i.). We conclude that contusive SCI triggers GI abnormalities with unique pathophysiology and pathology in different segments. Such GI disorders evolve continuously during the entire post-SCI period examined, and may require therapeutic development to target specific underlying mechanisms. Published by Elsevier Ltd on behalf of IBRO.

Key words: spinal cord injury, gastrointestinal dysfunction, rat, IJP, VIP, nNOS.

In humans, disturbances of gastrointestinal (GI) function are invariable sequelae in para- and quadriplegia after resolution of spinal shock, i.e. autonomic and somatic hypo or areflexia immediately following spinal cord injury (SCI), and are manifested in varied symptoms, including constipation and diarrhea (Stone et al., 1990). GI dysfunction in chronic SCI often leads to the consequence that emptying the bowels can occupy a significant portion of the patient's time and further jeopardize the quality of life. In fact, approximately 30% of people living with SCI consider bowel disorder to be a greater concern than either bladder or sexual dysfunction (Krogh et al., 1997). Medical problems associated with neurogenic bowel may include poorly localized abdominal pain, ulcer, difficulty or prolonged time with bowel evacuation, autonomic dysreflexia, bowel obstruction, bacteria infection, and other symptoms (Goetz et al., 2005). Furthermore, it is generally agreed that SCI detrimentally affects the gastric emptying (GE) in humans, with delayed GE in the chronic phase postinjury (p.i.) having been reported (Segal et al., 1987, 1995; Kao et al., 1998, 1999).

However, GI changes following traumatic SCI largely have not been studied in experimental settings; no studies have been reported using contusive injury models that most closely emulate pathophysiology derived from pathology commonly seen in clinical SCI (Bracken et al., 1990; Basso et al., 1995). Physiologic studies on animals have, nevertheless, shown that the GI system receives input from the CNS mainly through autonomic nerves and sends output through the parasympathetic nervous system via the dorsal motor nucleus of the vagus nerve (Pearson et al., 2007). The spinal reflex center, and the intrinsic activity of the GI smooth muscles and their interactions with the enteric nervous system can also partially program GI activity locally (Brading and Ramalingam, 2006). Because these types of activity may malfunction after SCI, neurogenic bowel and autonomic dysreflexia can occur in the

[1] S. K. and D. Y. contributed equally to this study.

*Correspondence to: Y. D. Teng, Department of Neurosurgery, Harvard Medical School/BWH/CHB, 75 Francis Street, PBB CA 138, Boston, MA 02115, USA. Tel: +1-617-525-8676; fax: +1-617-264-5216.

E-mail address: yang_teng@hms.harvard.edu (Y. D. Teng).

Abbreviations: A/*n*, numbers of animals/numbers of tissue samples; ANOVA, analysis of variance; BBB, Basso-Beattie-Bresnahan; BW, body weight; DNO, diethylenetriamine-nitric oxide; DRR, dye recovery rate; EFS, electrical field stimulation; eNOS, endothelial nitric oxide synthase; GE, gastric emptying; GI, gastrointestinal or gastrointestine; IJP, inhibitory junction potential; LNNA, L-N_{ω}-nitro arginine; MP, methylprednisolone; NANC, non-adrenergic non-cholinergic; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; O.D., optical density; p.i., postinjury; SCI, spinal cord injury; SP, substance P; TTX, tetrodotoxin; VIP, vasoactive intestinal polypeptide.

0306-4522/08$32.00+0.00 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2008.04.071

lack of proper upper level neural control and spinal afferent input. In rats, as in most mammalian animals, it is known that autonomic hyperreflexia is quickly established after high-level cord transection (Osborn et al., 1990), whereas the effect of a disconnection of the spinal cord from supraspinal centers on autonomic (parasympathetic and sympathetic) functions and its impact on the enteric intrinsic neurophysiology still remain unclear (Enck et al., 2006). Importantly, previous work on spinal cord transection showed that total T4–T5 severance in rats markedly inhibited GE, as well as GI and intestinal transit of liquid, during both the spinal shock and autonomic hyperreflexia phases (Gondim et al., 1999, 2001). We therefore hypothesized that contusion models of SCI may help to systematically characterize GI abnormalities resulting from clinical SCI, and reveal the underlying pathophysiological mechanisms. Such causal cellular and molecular events can then be targeted for developing novel therapies, and for assessing putative treatments valuable in ensuring maximum neurological improvement after SCI. We have now tested our hypothesis by investigating GI changes in post-T10–T11 contusion rats using liquid dye assay of transit, histopathology for depicting tissue atrophy, and mechanistic examinations on the relaxant non-adrenergic non-cholinergic (NANC) neurons in the myenteric plexus through electrophysiology and immunocytochemistry (Crist et al., 1991).

EXPERIMENTAL PROCEDURES

Surgical procedures and behavioral studies

Experiments were performed on 35 young adult female Sprague–Dawley rats (~2 months old, 200–220 g; Charles River Laboratories, Wilmington, MA, USA). Under a dissection stereomicroscope (Zeiss SV6; Carl Zeiss Microimaging, Thornwood, NY, USA), a 3 mm long laminectomy, encompassing the caudal end of T10 vertebra and the rostral end of T11 vertebra, was performed after anesthesia (ketamine, 75 mg/kg and xylazine, 10 mg/kg; i.p.), to expose the underlying spinal cord prior to the introduction of SCI. For SCI groups (total: $n=19$), a severe T10–T11 contusive injury was introduced by dropping the impounder rod (10 g) from a height of 50 mm, using the New York University (NYU) impactor (Gruner, 1992; Basso et al., 1996; Teng et al., 2004). The lesion zone was closed following SCI, and the animal was allowed to recover in a clean cage that was partially heated for 12 h after surgery. The bladders of SCI rats were evacuated twice daily during the entire study. The proper production of SCI was visually confirmed by the appearance of a bruise at the SCI site in all cases immediately following the weight drop, and further verified by our standardized behavioral and clinical evaluations on spinal shock, locomotion, spinal reflexes, and bladder dysfunction (Teng et al., 1999, 2004).

One day p.i. and weekly thereafter, behavioral analysis was performed by two observers blinded to the treatments to evaluate the level of hind limb function post-T10–T11 SCI. A battery of behavioral tests was carried out to rate performance of open-field locomotion based on the BBB (Basso-Beattie-Bresnahan) scale that ranges from 0 for complete paralysis to 21 for normal hind limb function (Basso et al., 1995; Teng et al., 1999, 2002), the ability to maintain body position on an inclined plane, and contact righting reflex and spinal cord-mediated hind limb withdrawal to pain (i.e. a brief pinch to the toe pad by forceps tips; Gale et al., 1985). Behavioral tests were performed at the same time each day and graded by the same observers. For the inclined plane test, the highest degree of inclination was defined as being that at which the animal could maintain its position for 5 s in two separate trials (Teng et al., 2002). Naïve rats and rats post–"sham surgery" were also tested to ensure they were free of any neurological dysfunction.

Experimental protocol and statistical analysis

The experiments were conducted according to a randomized block design. The size of the experimental groups is comparable to those of our previous studies that were determined on the basis of power analyses (Teng et al., 2003). Four different treatments were included in this study: (a) "naïve" control group (rats received no surgery; $n=8$); (b) "sham surgery" control group (rats underwent laminectomy only; $n=8$); (c) "SCI-48 h" group ($n=11$) included rats that survived 48 h after SCI before tissue collection; and (d) "SCI-4 weeks" group ($n=8$) consisted of rats that were terminated 4 weeks p.i. Of all the experimental animals, five rats from each group were randomly assigned for dye recovery assays, 3 "sham surgery," SCI-48 h and SCI-4 weeks rats for immunocytochemical analysis, and 3 "naïve control" and SCI-48 h rats for electrophysiological studies. All experimental procedures were reviewed and approved by our institutional animal care and use committees in accordance to published guidelines.

GI transit data were analyzed statistically using the one-way analysis of variance (ANOVA) followed by independent Student's *t*-test to compare the differences in Phenol Red recovery along the different segments between the various groups. The electrophysiology data were analyzed using the paired Student's *t*-test to determine whether a difference has occurred between the naïve and SCI-48 h groups in the antrum and duodenum. Histopathologic data were assessed using the repeated-measures ANOVA and independent Student's *t*-test to detect the differences between the naïve and SCI groups in each segment of the GI. For comparison of immunoreactivity intensities, data were analyzed using the one-way ANOVA with Tukey error protection and two-tailed independent *t*-test to detect the differences between the "sham surgery" control and SCI groups in each GI segment. Statistical analysis was done with the Analyze-it™ Statistics (Analyze-it Software Ltd., Leeds, UK) for Excel™ (Microsoft, Redmond, WA, USA). All values are expressed as mean±S.E.M. Use of the term "significant" in the text indicates that statistical testing was performed and $P<0.05$.

Measurement of GI transit

For GI transit measurements, we used a modified version of the methods described previously (Reynell and Spray, 1956; Gondim et al., 2001; de Rosalmeida et al., 2003). Briefly, rats ($n=5$/group) were deprived of food 24 h prior to the evaluation (water was allowed *ad libitum* until 2 h before the measurement). We used 1.5 ml of test meal containing a non-absorbable marker (0.5 mg/ml Phenol Red solution in 5% glucose) to track the GI transit. The test liquid was given to the animals orally by gavage feeding. After 10 min, all animals were killed before surgical removal of the GI tract (Gondim et al., 2001; Sener et al., 2006). The stomach was clamped at the cardia and pylorus prior to the surgical removal, and the small intestines from the gastroduodenal junction to the cecum were divided into the duodenum, jejunum, and ileum. Segmental tissue volume was determined by measuring the displacement of 10 ml of 0.1 N NaOH after adding the tissue to the solution in a 50 ml falcon centrifuge tube. The segments were then cut into small pieces and homogenized for 30 s. After 20 min settling at room temperature, the supernatant was centrifuged for 10 min (2800 r.p.m.; Eppendorf 5804R; Eppendorf North America, Westbury, NY, USA). We further precipitated the proteins in the consequent supernatant (5 ml) with 0.5 ml trichloroacetic acid (20% w/v) with subsequent centrifuge precipitation (2800 r.p.m.×20 min). The final supernatant (3 ml) was added into 4 ml