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ORIGINAL ARTICLE

Stimulation of the sacral nerve reduces gut bacterial translocation and endotoxemia caused by acute spinal cord injury in rabbits

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Study design: Disturbance of gastrointestinal motility following acute spinal cord injury complicated with paraplegia can lead to bacterial overgrowth in the gastrointestinal tract, and increase the incidence of bacterial translocation. Experiments in a New Zealand rabbit model of acute spinal cord injury were performed.

Objective: This study was designed to determine if the electrical stimulation of the sacral nerve prevents gut-origin bacterial translocation and endotoxemia in an animal model of acute spinal cord injury.

Settings: Xiangya Hospital, Central South University, Hunan, PR China.

Methods: New Zealand rabbits were divided into three groups: Control group, Paraplegic Vehicle group without sacral nerve stimulation and Paraplegic Experiment group with sacral nerve stimulation. Blood and multiple organs were collected for bacterial cultures and endotoxin determination at 24, 48 and 72 h following spinal cord injury. The histology and ultra-structural features of the organs were studied.

Results: Bacterial translocation and endotoxemia were observed in all animals with acute spinal cord injury. Sacral nerve stimulation increased defecation, decreased endotoxin levels and bacterial translocation and improved the morphology of the organs.

Conclusion: After acute spinal cord injury, stimulation of the sacral nerve reduced gut bacterial translocation and endotoxemia.

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Keywords: spinal cord injury; paraplegia; sacral root stimulation; bacterial translocation

Introduction

Acute spinal cord injury (ASCI) associated with paraplegia results in constipation due to delayed left colonic and rectosigmoid transit, which can lead to gut bacterial translocation and endotoxemia.[1] Bacterial translocation and endotoxemia may involve other mechanisms,[2] but the disturbance of gastrointestinal motility resulting from ASCI is one of the mechanisms. Hence, the improvement of intestinal motility is important for preventing subsequent bacterial translocation and endotoxemia.

The technique of sacral nerve stimulation (SNS) has been used in the treatment of urinary incontinence[3] and faecal incontinence.[4] Although some studies have shown improved intestinal tract motility by stimulation of the sacral nerve for the treatment of constipation,[5] the prevention of gut-origin bacterial translocation and endotoxemia, particularly in the condition of ASCI, has not been studied.

This study was designed to determine if SNS may reduce gut bacterial translocation and endotoxemia resulting from ASCI.

Materials and methods

Animal care and use

Seventy-two New Zealand rabbits of either sex, weighing 2.0–2.5 kg, were purchased from Hunan Laboratory Animal Technology Co. Ltd., and were kept in separate cages under the same living conditions for a week before the operation. All animals received care in compliance with the Convention on Animal Care, and the study was approved by the Ethics Committee of Central South University, China.

The animals were randomly assigned to the Control group (CG: $n = 24$), Paraplegic Vehicle group (PV: $n = 24$) without sacral nerve stimulation and Paraplegic Experiment group (PE: $n = 24$) with sacral nerve stimulation. Each group was divided into 24, 48 and 72 h post-injury subgroups (eight animals in each subgroup).

Operation procedure

ASCI was produced using previously reported methods.[6] Briefly, under general anesthesia, the rabbit was placed in a prone position to extend the thoracic spinal column and increase the width of interspaces between the T5–T7 spinal processes. All animals in both the PV and PE groups underwent a clip compression injury at T6. The hind limbs had no movement, as measured by the Tarlov scale.[7] Animals in the Control group underwent no compression injury.

Electrode implantation and sacral nerve stimulation (SNS) protocol

The right 3rd sacral nerve root was found, and one needle electrode was positioned in the right sacral foramina. The wires connecting the electrodes went through the subcutaneous tissue, exited the back of the animal, and connected to the stimulator (Model 3625, Minneapolis, MN, USA). Correct placement of the needle electrodes was confirmed by the contractile response of muscles in the pelvic floor, sphincters and hind limbs.

The electrode was implanted in the PV and PE groups, and no electrode was implanted in animals of the Control group.

The animals in the PE group underwent 2-h periods of SNS, twice a day, at 0800–1000 hours and 1800–2000 hours. The stimulation frequency was 14 Hz with a pulse width of 300 μs. Our preliminary experiments indicated that 2 h were the optimal stimulation period for maximum colorectal activation. Animal in the PV group did not undergo SNS.

Sample schedule and materials collected

Animals were killed by exsanguination from the portal vein at 24, 48 and 72 h after ASCI. Samples of blood, liver, spleen and mesenteric lymph nodes were obtained at killing.

Measurements

Endotoxin determination. Blood sampled from the portal vein was centrifuged. The plasma was preserved at $-70\,^{\circ}\text{C}$. Endotoxin in the plasma was determined using an endotoxin assay kit (GenScript, The Biology CRO, USA). In brief, standards, samples and limulus amebocyte lysate reagent were dispensed into endotoxin-free vials. Limulus amebocyte lysate was added to each vial, thoroughly mixed and the vials incubated at $37\,^{\circ}\text{C}$. Chromogenic substrate solution was added to each vial, mixed well and incubated at $37\,^{\circ}\text{C}$. Color stabilizer was added, mixed well and absorbance read.

Bacterial culture. Bacterial culture was performed following the method reported previously.[1] In brief, blood and specimens of the liver, spleen and mesenteric lymph nodes were obtained under aseptic manipulation and inoculated into bacteceaobic peds-plus flasks. When bacterial growth was found, the specimen was removed from the culture flask and placed on blood agar culture medium at $37\,^{\circ}\text{C}$. The intestinal contents were directly inoculated into blood agar culture medium. After 24 h of culture, the colony morphology was examined, and specimens were inoculated into the typing systems (ATB, Expression, Biomerieux, France). *Streptococcus*

was identified in 4 h. *Enterobacter* and *staphylococcus* were identified in 24 h.

Histological and ultrastructural studies. Liver, spleen, small intestine and mesenteric lymph node tissue samples were prepared for histology. Tissue specimens for light microscopy were fixed in 10% neutral-buffered formalin and embedded in paraffin. These samples were sliced in thickness at about 5 μm with a cryostat, 6–10 slices prepared for every sample, and stained with hematoxylin and eosin. The slices were rinsed with phosphate-buffered saline.

Ultra-structural studies of the jejunum were performed by electron microscopy. Specimens were cut into 2 mm cubes, prefixed in a mixture of 4% glutaraldehyde and phosphate buffer for 2 h, re-fixed in a solution of 1% osmium tetroxide, gradually dehydrated by alcohol and acetone, saturated and embedded. Ultra-thin sections were prepared, stained with uranyl acetate and lead citrate and then examined by electron microscopy.

Stool volume. The total volume and weight of the contents of the colon (stool) was determined at killing.

Statistical analysis

All experimental data were analyzed using a statistical software package, SPSS11.0, and were expressed as mean ± s.d. Student's t-test was performed, and a $P < 0.05$ was considered statistically significant.

Results

Bacterial translocation

Bacterial growth in the blood, liver, spleen and mesenteric lymph nodes was found in all animals with ASCI. Bacterial growth was significantly lower in the PE group with sacral nerve stimulation (SNS) than in the PV group without the stimulation (Table 1). Translocation bacteria were mainly *Bacillus coli*, *Enterobacter cloaca*, *Escherichia coli* and *Enterococcus faecalis*.

Endotoxin in the blood

Endotoxin in the blood was detected in all animals with ASCI. The level of endotoxin was decreased in the PE group with SNS compared with the PV group without the stimulation (Table 2).

Stool volume and weight

Contents of the colon were increased in all animals with ASCI. The volume of stool was lower in the PE group undergoing SNS compared with the PV group without the stimulation. The volume of stool in the PV group gradually increased from 24 to 72 h, whereas in the PE group undergoing stimulation, the stool weights were similar at the three time-points (Table 3).

Histology

Normal histology was found in the histology of the liver, small intestine, spleen and mesenteric lymph nodes of the

Table 1 The number of animals with bacterial translocation

Groups	Lymph node	Blood	Liver	Spleen
CG	0/24	0/24	0/24	0/24
PE	6/24*	7/24*	8/24*	6/24*
PV	15/24**	16/24**	15/24**	10/24**

CG, Control group; PE, Paraplegic experiment group with sacral nerve stimulation; PV, Paraplegic vehicle group without sacral nerve stimulation.
*P<0.05, comparison of PE to PV.
**P<0.01, comparison of PV to CG.

Table 2 Levels of endotoxin in blood at different time-points

Group	24 h	48 h	72 h
CG	0.10 ± 0.11	0.09 ± 0.14	0.11 ± 0.11
PE	0.89 ± 0.11*	1.06 ± 0.12*	1.09 ± 0.05*
PV	1.21 ± 0.25**	1.43 ± 0.77**	1.69 ± 0.70**

PE, Paraplegic Experiment group with sacral nerve stimulation; PV, Paraplegic Vehicle group without sacral nerve stimulation.
*P<0.05, comparison of PE to PV.
**P<0.01, comparison of PV to CG.

Table 3 Weight of stool in the colon at different time-points

Groups	24 h	48 h	72 h
CG	30.2 ± 10.5	32.1 ± 11.2	32.3 ± 9.3
PE	36.4 ± 5.8*	41.6 ± 43.4*	40.3 ± 18.7*
PV	62.1 ± 9.6**	108.4 ± 20.9**	119.1 ± 28.1**

PE, Paraplegic experiment group with sacral nerve stimulation; PV, Paraplegic Vehicle group without sacral nerve stimulation.
*P<0.001, comparison of PE to PV.
**P<0.001, comparison of PV to CG.

Control group animals (Figures 1–4a). Alterations of the histology of the liver, small intestine, spleen and mesenteric lymph nodes were observed in both the PV and PE groups. At 24 and 48 h post-ASCI, slightly more abnormal findings were observed in the PE group compared with the PV group, but the differences between the groups were marked at 72 h post-ASCI. Representative sections of these organs are shown in Figures 1–4. A summary of the observations of the histology of these organs is as follows:

The liver. In the PV group without SNS, congestion in converge duct region was observed at 24 h; and, congestion in central veins, swelling of liver cells at 48 h. Congestion in central vein and converge duct region, severe swelling, edema and punctiform necrosis of liver cells (Figure 1b) was observed at 72 h. In the PE group with SNS, slight congestion in converge duct region was observed at 24 h. Congestion in central veins, slight swelling of liver cells were observed at 48 h. Congestion in central veins, slight edema of liver cells and no punctiform necrosis was seen (Figure 1c) were observed at 72 h.

The jejunum. In the PV group without SNS, hyperemia of blood vessel and infiltration of inflammatory cells were
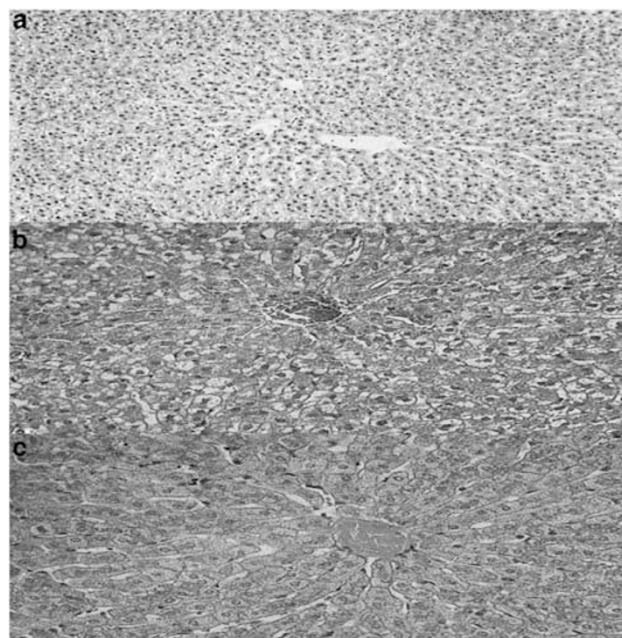

Figure 1 Liver histology 72 h after acute spinal cord injury. Specimens of the liver were fixed in neutral-buffered formalin, sections stained with hematoxylin and eosin, and examined by light microscopy. (**a**) Control group. (**b**) Paraplegic vehicle group without sacral nerve stimulation. (**c**) In the Paraplegic experiment group with sacral nerve stimulation.
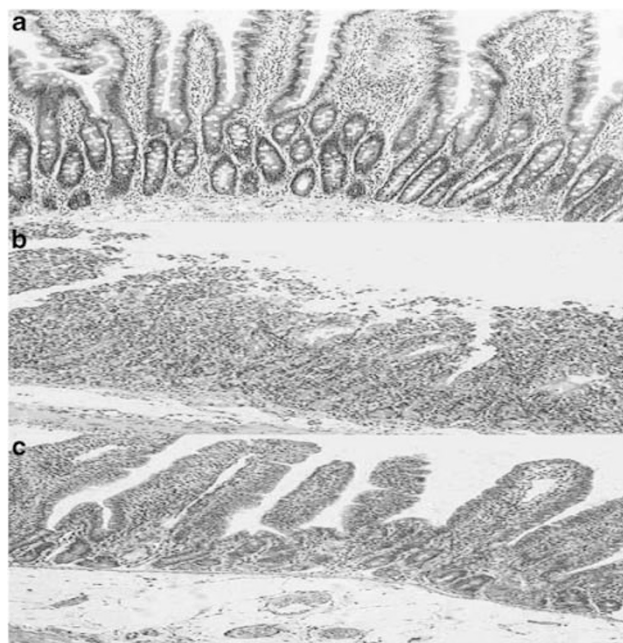

Figure 2 Histology of the jejunum. Specimens of the jejunum were fixed in neutral-buffered formalin, sections stained with hematoxylin and eosin, and examined by light microscopy. (**a**) Control group. (**b**) Paraplegic vehicle group without sacral nerve stimulation. (**c**) Paraplegic experiment group with sacral nerve stimulation.

observed at 24 h; hyperemia of blood vessel, slight fibrinoid exudation and infiltration of inflammatory cells at 48 h; and 72 h; hyperemia of blood vessel, edema of submucous tissue,
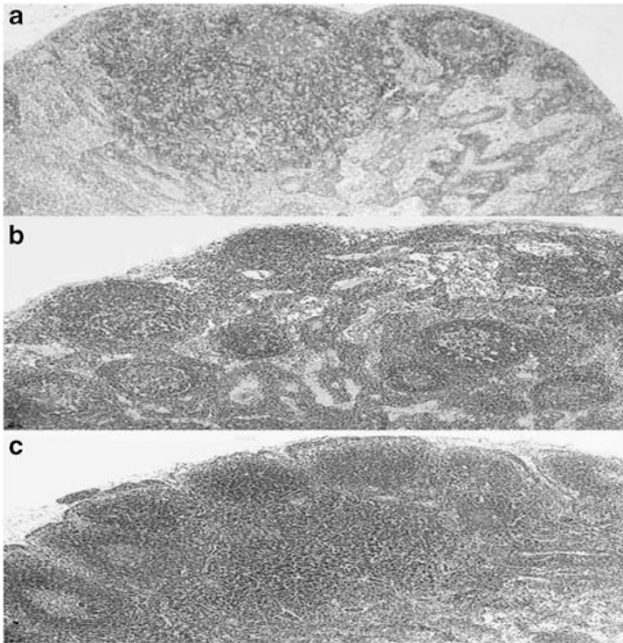
Figure 3 Histology of the mesenteric lymph node. Specimens of the mesenteric lymph node were fixed in neutral-buffered formalin, sections stained with hematoxylin and eosin, and examined by light microscopy. (**a**) Control group. (**b**) Paraplegic vehicle group without sacral nerve stimulation. (**c**) Paraplegic experiment group with sacral nerve stimulation.
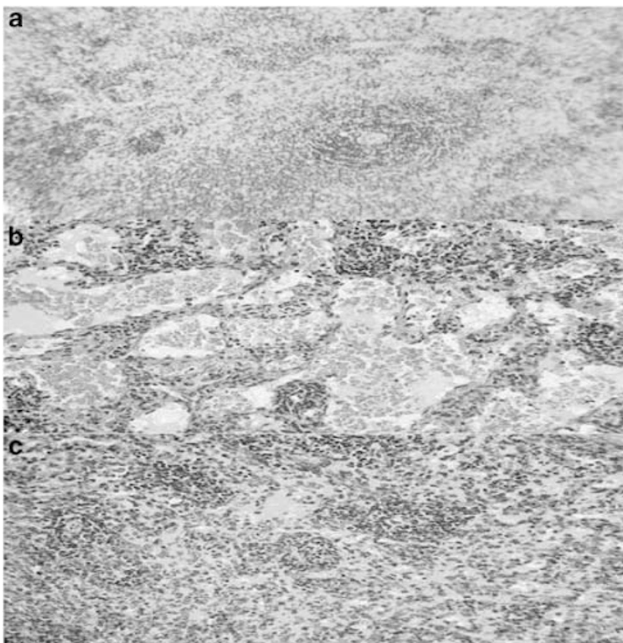

Figure 4 Histology of the spleen. Specimens of the spleen were fixed in neutral-buffered formalin, sections stained with hematoxylin and eosin, and examined by light microscopy. (**a**) Control group. (**b**) Paraplegic vehicle group without sacral nerve stimulation. (**c**) Paraplegic experiment group with sacral nerve stimulation.

atrophy and disintegration of the villus, necrosis of mucosal epithelial cells, infiltration of inflammatory cells and fibrinoid exudation (Figure 2b). In the PE group with SNS;

hyperemia of blood vessel and slight infiltration of inflammatory cells were observed at 24 h. Hyperemia of blood vessel, slight fibrinoid exudation and infiltration of inflammatory cells at 48 h. Hyperemia of blood vessel, slightly fibrinoid exudation and infiltration of inflammatory cells at 72 h (Figure 2c).

The mesenteric lymph node. In the PV group without SNS dilation of lymph vessel and proliferation of cortex were observed at 24 h. Intumescence of lymph node, dilation of lymph vessel and proliferation of cortex at 48 h. Increase of lymph node, dilation of lymph vessel and proliferation of lymphatic cells in cortical zone at 72 h (Figure 3b). In the PE group with SNS, slight dilation of lymph vessel at 24 h. Slight dilation of lymph vessel and proliferation of cortex at 48 h. Slight increase of germinal center and proliferation of lymphatic cells in the cortical zone at 72 h (Figure 3c).

The spleen. In the PV group without SNS, mild congestion in parenchyma of spleen at 24 h. Congestion in parenchyma and thickening of white pulp at 48 h. Severe proliferation and congestion of white pulp, proliferation of lymph tissue, compression of splenic cord and unsharpness of acinus lienalis at 72 h (Figure 4b). In the PE group with SNS, no congestion in parenchyma of spleen at 24 h. Slight congestion in parenchyma and thickening of white pulp at 48 h. Slight proliferation and congestion of white pulp; no change of splenic cord and acinus lienalis at 72 h (Figure 4c).

Ultrastructure of the jejunum
The ultrastructural features of the jejunum were examined by electron microscopy, and representative sections are shown in Figure 5. A summary of the observations of the jejunum is as follows:

In the PV group without SNS at 24 h, the epithelial cells developed slight edema with wide epithelial cell interspaces. At 72 h, the epithelial cells showed severe edema, necrosis of the epithelial cells was seen, and interspaces became very wide. Edema, breaks and exfoliation were seen in the microvilli (Figure 5a). In the PE group with SNS at 24 h, the epithelial cell developed slight edema with normal epithelial cell interspaces. At 72 h, the microvilli were loosely distributed (Figure 5b).

Discussion

The primary mechanisms promoting bacterial translocation are the disruption of the ecologic gastrointestinal equilibrium, allowing intestinal bacterial overgrowth; increased permeability of the intestinal mucosal barrier and deficiencies in host immune defense.[2] These mechanisms may synergistically promote the systemic spread of indigenous translocated bacteria to cause lethal sepsis.[8,9] Bacteria or endotoxin crossing the mucosal barrier can trigger an augmented immune response, so that the gut becomes a proinflammatory organ, releasing chemokines, cytokines and other proinflammatory intermediates. These intermediates may affect the local and systemic immune systems, and
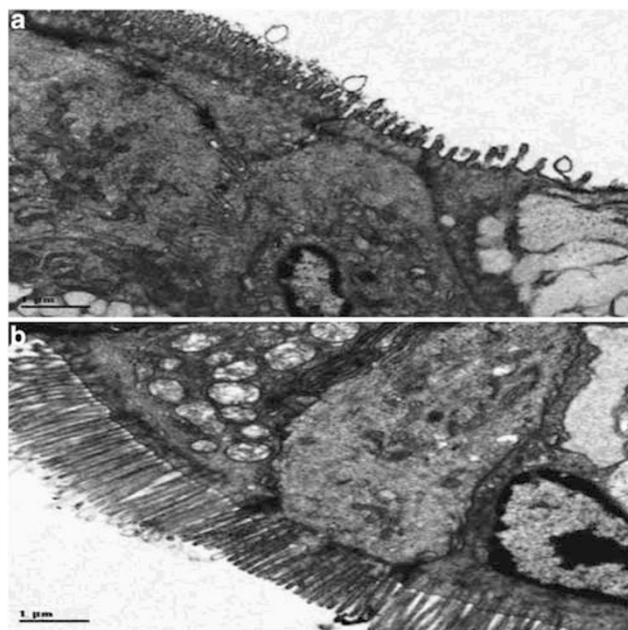
Figure 5 Ultrastructural structure of the ileum observed by electron microscopy 72 h after acute spinal cord injury. Ultra-thin sections were prepared, stained with uranyl acetate and lead citrate and examined by electron microscopy. (**a**) Paraplegic vehicle group without sacral nerve stimulation (magnification = ×8900). (**b**) Paraplegic experiment group with sacral nerve stimulation (magnification = ×8900).

even result in systemic inflammatory response syndrome and multiple organ dysfunction syndromes.

The intestinal tract is an acute reactive target organ after trauma. An initial insult results in hypoperfusion in the gut, which becomes a major site of proinflammatory factors. Subsequent reperfusion leads to an ischemia-reperfusion injury to the intestine with a resultant loss of gut barrier function and an ensuing enhanced gut inflammatory response.[9] In addition, after ASCI paraplegia, gastrointestinal motility disturbance resulting from the dysfunction of the autonomic nervous system occurs, characterized by a delay of gastric emptying and gastrointestinal transit of liquid.[10] The inhibition of gastrointestinal motility after spinal cord transection seems to involve vagal and possibly splanchnic pathways.[10] Intestinal motor abnormalities are responsible for enteric bacterial overgrowth, and attenuated migrating motor complex activity results in bacterial overgrowth.[11] Therefore, constipation or obstruction caused by ASCI leads to bacterial overgrowth, and destruction of the intestinal structures can be observed by histology and cytology.[1,12]

The destruction of the intestinal histology and cytology leads to microorganism translocation occurring from the intestine to the peritoneal cavity and other organs.[13] The increased permeability seen with intestinal obstruction is because of the destruction of intestinal mucous barrier and the obstruction of defecation.[1] Intestinal obstruction is accompanied by suppression of the immunosecretory and motor evacuatory functions of the intestine, as well as by pronounced bacterial contamination. On this basis, endotoxin in the blood and translocated bacteria aggravate the

injury of the organs or systems. In addition, ASCI may delay gastrointestinal transit,[10] colonic transit time and impair anal relaxation.[13] The delay in colonic transit caused by the denervated colon following ASCI is one of the reasons for constipation,[14] whereas SNS promotes colon mobility, and increases luminal transport and defecation.[15,16]

Our studies have further confirmed that endotoxemia and bacterial translocation from the gut occurs after ASCI. These alterations are in part consistent with the findings reported by Liu and colleague.[1] Even though their studies have concluded that antibiotics should be administered to paraplegic patients with ASCI to prevent potential bacterial translocation,[1] the possibility of dual infection and side effects from these drugs should be considered.

This study showed that the electrical stimulation of the 3rd sacral nerve root promoted intestinal motility, which increased luminal transport and defecation, and decreased bacterial translocation and endotoxin. In addition, the positive impact of the electrical stimulation of the sacral nerve root was evidenced by improvement in the histological findings in the liver, intestine, spleen and mesenteric lymph nodes. These histological findings confirmed that the electrical stimulation of the sacral nerve root improved the morphology of the digestive system, and produced parallel functional changes, such as an increase in intestinal motility, and a decrease in bacterial translocation and endotoxin.

The protective effect of nerve stimulation on gut damage is related to the mechanism of the innervation of the sigmoid colon and rectum. It has been shown that the 3rd sacral nerve mainly innervates the sigmoid colon and rectum, and that stimulation of the 3rd sacral nerve increased pressure,[17] because the 3rd sacral nerve is primary in affecting the function of sphincter ani externus.[18]

Electrical stimulation of the 3rd sacral nerve has been shown to induce pan-colonic propagating pressure waves and significantly increase pan-colonic antegrade propagating sequence frequency, which increased stool frequency and quantity.[15] Therefore, electrical stimulation of the 3rd sacral nerve promotes intestinal motility, and improves the function of the digestive tract. The stimulation increases luminal transport and defecation, and decreases the levels of endotoxin and the rate of gut bacterial translocation.

This study showed that bacterial growth in the blood, liver, spleen and mesenteric lymph nodes was found in the animals with ASCI. In addition, wider interspaces of intestinal epithelial cells were found in the animal with ASCI. In contrast, the interspaces of intestinal epithelial cells were reduced and bacterial growth was decreased in the animals undergoing sacral nerve stimulation, suggesting that bacteria in the organs originate in the gut, and sacral nerve stimulation decreases bacterial translocation through improvement of epithelial cell interspaces.

Conclusion

Our studies suggested that electrical stimulation of 3rd sacral nerve root promoted intestinal motility, and improved the function and morphology of the damaged digestive tract

after ASCI. The stimulation increased luminal transport and defecation, and decreased endotoxin and gut bacterial translocation.

Conflict of interest

The authors declare no conflict interest.

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RETRACTION

Retraction: Stimulation of the sacral nerve reduces gut bacterial translocation and endotoxemia caused by acute spinal cord injury in rabbits

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The authors of the above article would like to retract it.

All the authors of the above article would like to retract this paper. The paper contains inaccuracies in four figures (Figures 2b and c, and 5a and b), which, although similar to the original images and containing the same message, are not the original pictures referring to the experiments performed. The authors apologize to the readers, reviewers and editors of *Spinal Cord* for publishing these erroneous images.