Electroacupuncture modulates the intestinal microecology to improve intestinal motility in spinal cord injury rats

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Introduction

Spinal cord injury (SCI) is a devastating condition that currently has no cure. Patients with SCI often experience neurological impairment and secondary complications, such as colorectal, bladder and sexual dysfunction (Lynch et al., 2001; Anderson, 2004). Over two-thirds of all patients with SCI will develop symptoms of intestinal dysfunction, such as faecal incontinence or constipation, leading to neurogenic intestinal dysfunction (Bryce et al., 2021). Intestinal dysfunction not only impairs the dignity and quality of life of patients but also aggravates neurological damage (White and Holmes, 2018). Indeed, some researchers believe that the recovery of intestinal function is even more important than the ability to walk.

Recent clinical research on electroacupuncture (EA) treatment for SCI has shown remarkable preliminary results, and the treatment is believed to have potential curative effects on motor dysfunction, hypertonia, neuralgia and other aspects after SCI (Fan et al., 2018). For SCI patients with constipation, EA therapy can promote intestinal motility and improve symptoms without obvious toxic and side effects. For example, a single-blinded randomized controlled trial found that acupuncture at Zusanli has a significant adjustment effect on gastrointestinal motility disorders in SCI patients (Jin et al., 2015). EA may also improve intestinal movement by regulating the daily rhythmicity and increasing the expression of the circadian rhythm of colonic Per2 (Cheng et al., 2016). Guo et al. (2016) found that EA at Zusanli (ST36) ameliorates neurogenic bowel dysfunction following SCI in rats by downregulating colonic neuronal nitric oxide synthase.

Summary

Spinal cord injury (SCI) is a disease involving gastrointestinal disorders. The underlying mechanisms of the potential protective effects of electroacupuncture (EA) and 5-hydroxytryptamine (5-HT) system on SCI remain unknown. We investigated whether EA improves gut microbial dysbiosis in SCI and regulates the 5-HT system. 16S rDNA gene sequencing was applied to investigate alterations in the gut microbiome of the rats. Faecal metabolites and the expression of the 5-HT system were detected. EA and faecal microbiota transplantation (FMT) treatment facilitated intestinal transmission functional recovery and restored the colon morphology of SCI rats. The composition of the intestinal microbiota, including numbers of phylum Proteobacteria, class Clostridia, order Bacteroidales, and genus Dorea, were amplified in SCI rats, and EA and FMT significantly reshaped the intestinal microbiota. SCI resulted in disturbed metabolic conditions in rats, and the EA and FMT group showed increased amounts of catechin compared with SCI rats. SCI inhibited 5-HT system expression in the colon, which was significantly reversed by EA and FMT treatment. Therefore, EA may ameliorate SCI by modulating microbiota and metabolites and regulate the 5-HT system. Our study provides new insights into the pathogenesis and therapy of SCI from the perspective of microbiota and 5-HT regulation.
However, the regulatory pathway of EA to the gastrointestinal dynamical system remains unknown.

Increasing evidence suggests that an abnormal intestinal microbial community is involved in the pathogenesis and clinical symptoms of SCI (Gungor et al., 2016). Existing theories indicate that damage to the nerve conduction pathway between the brain and the gut of SCI patients decreased intestinal motility, and alterations in the secretion and permeability of the mucosal epithelium will lead to cause the displacement and imbalance of the intestinal flora in patients with SCI and disrupt the dynamic balance of gut microbes; the imbalance of intestinal flora could aggravate bowel dysfunction and cause persistent pathological changes in such patients (Kigerl et al., 2016, 2018). Therefore, the establishment of a benign regulation mechanism of the intestinal flora of patients with SCI is of great significance for the recovery of intestinal function. EA and microbiota-targeted techniques, such as faecal microbiota transplantation (FMT), can promote gut microbiota remodelling, which may play a role in SCI (Han et al., 2021; Jing et al., 2021). However, the mechanism of gut microbiota remodelling in SCI improvement is unclear.

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) system may be the underlying mechanism of microbial regulation of host behaviour (Chen et al., 2020). The 5-HT system mainly consists of 5-HT, 5-HT receptor and the serotonin selective reuptake transporter (SERT). Blocking 5-HT3R and 5-HT4R, the excitatory 5-HT receptor will lead to decreased gastrointestinal motility, whereas using agonists promotes gastric emptying (Morita et al., 2013; Hussain et al., 2017). The 5-HT pathway also includes tryptophan hydroxylase (TPH-1) and Toll-like receptors (TLR). TPH-1 is the rate-limiting enzyme involved in the majority of 5-HT synthesis in the gastrointestinal tract (McKinney et al., 2001). The 5-HT system is an important mediator of multiple regulatory effects on intestinal function. Intestinal bacteria from healthy individuals and mice, especially spore-forming bacteria and their metabolites short-chain fatty acids, have been found to contribute to increased 5-HT synthesis and release by regulating TPH-1 transcription (Reigstad et al., 2015). Therefore, the 5-HT system may be the underlying mechanism through which EA or FMT treatment achieves microbial regulation of SCI; however, this hypothesis has yet to be confirmed.

In the present study, we established a rat model of traumatic SCI and then subjected rats in the treatment group to EA therapy. The faecal suspension of EA-treated SCI rats was collected for analysis. Pathological changes and the intestinal function of rats in each group were detected, and faeces were collected for 16S rDNA sequencing and metabolite profiling analyses. Finally, the effects of EA and FMT on the expression of the 5-HT system were evaluated. Our study aims to investigate whether EA improves SCI by correcting the intestinal microbiota and determine whether this process involves 5-HT regulation.

**Results**

**EA ameliorates the intestinal function and pathology of SCI rats**

In this study, the BBB score of all rats before operation was 21. No rats died after modelling, and the 24 h BBB score was 0, which indicates model success. The surviving SCI rats were then assigned to undergo EA or FMT treatment (Fig. 1A). The defecation volume collected over 24 h and faecal water content of rats in the SCI group decreased, and the efflux time of the first black stool was prolonged (Fig. 1B and D). The EA and FMT groups showed significant differences compared with the SCI group in terms of these three indicators (Fig. 1B and D), as well as unobstructed defecation. The abdominal withdraw reflex (AWR) scores showed no significant differences among groups in all three condition (Fig. 1E), which suggests that EA and FMT have no effect on intestinal sensitivity function. H&E staining indicated that SCI induces remarkable pathological changes in colon tissues characterized by colonic mucosal erosion, fewer glands, interstitial oedema, inflammatory cell infiltration in the lamina propria and prominent atrophy of the intestinal wall and muscular layers; EA and FMT treatment clearly restored the morphology of the colon after SCI (Fig. 1F). Therefore, EA can ameliorate the intestinal function and pathology of SCI rats.

**EA and FMT treatment alters the dysbiosis of the gut microbiome in SCI rats**

Dysbiosis of the gut microbiome is associated with SCI. Therefore, 16S rDNA gene sequencing analysis of faecal samples was conducted to evaluate the effects of EA and FMT from EA donor rats on the dysbiosis of the gut microbiome in SCI. In this study, a total of 830 operational taxonomic units (OTUs) with 97% similarity with 514 OTUs were recognized across the 40 samples obtained (Fig. S1A). The upward trend at the end of the species accumulation and dilution curves flattened (Fig. 2A, Fig. S1B), thereby indicating that most OTUs had been captured, that the sample size was sufficiently large to cover the majority of the microbial diversity information, and that only a few new species would be discovered by increasing the sample size. Rank abundance analysis also revealed that the richness and evenness of the microbiological compositions among the groups was similar (Fig. 2B). A significant reduction in α-diversity was observed in SCI rat compared with the sham group.
on the basis of the observed species and Chao1 indices, but no significant difference in Shannon diversity was noted (Fig. 2C and D). These findings suggest that SCI remarkably inhibits gut microbial community richness but does not affect the distribution of the abundance of the bacterial species. The extent of the similarity of gut microbial communities among the four groups was measured using principal coordinates analysis (PCoA) based on weighted UniFrac distances at the OTU level. The results showed that the intestinal flora of the sham and the three groups were significantly separated (ANOSIM value not show here). Moreover, the intestinal flora were significantly different between the SCI and EA groups (ANOSIM R = 0.301, P = 0.005) and highly significantly different between the SCI and FMT groups (ANOSIM R = 0.432, P = 0.001). No significant change in intestinal flora between the EA and FMT groups (ANOSIM R = 0.084, P = 0.098; Fig. 2E–F) was observed, which suggests that the overall structures of the microbial communities among the four groups were significantly altered. The β-diversity analysis also confirmed significant differences in microbial communities among the groups based on the weighted-Wilcoxon (Fig. S1C). The results collectively show that SCI significantly changes the gut microbial richness, diversity and composition and that EA and FMT treatment reverses this alteration.

EA and FMT treatment affects the abundance of certain bacteria in SCI rats

Differential relative abundance analyses at the phylum level indicated a significant increase in Proteobacteria in the SCI group, which significantly decreased after EA and FMT treatment (Fig. 3A). Whereas the predominance of Firmicutes decreased in the SCI microbiota (Fig. 3A). Furthermore, at the class level, the relative abundance of Gammaproteobacteria in Proteobacteria, Erysipelotrichia and Clostridia in Firmicutes significantly increased in the SCI group and decreased after EA and FMT treatment, while FMT demonstrated no significant effect on the abundance of Erysipelotrichia and Clostridia (Fig. S1D). At the genus level, the relative abundance of unidentified Enterobacteriaceae in Proteobacteria, Dorea, Allobaculum and Blautia in Firmicutes significantly increased after SCI modelling but decreased after EA and FMT treatment; however, Allobaculum and Blautia did not respond to FMT stimulation (Fig. S1E).

The cladogram of LEfSe comparisons based on the relative abundance identified 510 taxa (16 phyla, 22 classes, 40 orders, 70 families, 151 genera and 211 species) that differed among the four groups (Fig. 3B). Significant enrichments in class Gammaproteobacteria,
species *Blautia glucerasea* and genus *Dorea* were identified in SCI rats, while class *Clostridia*, order *Clostridiales*, genus *Blautia* was significantly abundant in FMT rats (Fig. 3C). These findings suggest that SCI is mainly related to Gammaproteobacteria dysbiosis, while FMT is mainly related to *Clostridia*. Phylum *unidentified_Bacteria*, class *unidentified_Bacteria*, order *Campylobacterales*, family *Helicobacteraceae*, genus *Helicobacter*, species *Helicobacter_rodentium* and phylum *Bacteroidetes*, class *Bacteroidia*, order *Bacteroidales*, family *Bacteroidaceae*, genus *Bacteroides*, species *Bacterium_P3* were identified in faecal samples from EA-treated rats (Fig. 3C), thus suggesting that improvement of SCI by EA may be mainly related to the restoration of the abundance of orders *Campylobacterales* and *Bacteroidales*. These speculations were further supported by Metastat analysis, which revealed differences in gut microbial communities among the four groups at five levels. As shown in Fig. S2, the abundance of *Clostridia*, *Clostridiales* and *Blautia glucerasea* significantly increased in the SCI group, significantly decreased after EA treatment, and showed no change following FMT. By contrast, the abundance of *Campylobacterales*, *Helicobacteraceae* and *Helicobacter* significantly decreased in the SCI group and significantly increased after EA and FMT treatment.

### Intestinal microbial metabolites

Certain end-products of gut microbial fermentation can enter the blood and affect the physiological functions of the central nervous system of the host (Mitchell *et al.*, 2011; Sharon *et al.*, 2016). To detect the potential metabolites involved in the regulation of SCI recovery, we examined the faecal metabolome of rats in each group. Significant differences between the SCI and sham groups and between the sham and EA groups (Fig. 4A) were noted. This finding indicates that SCI causes serious metabolite disorders and that EA regulation may be mediated by other key metabolites, rather than principal components in SCI rats. Supervised OPLS-DA further revealed that the SCI and sham groups could be clearly distinguished (Hotelling’s T-squared ellipse; Fig. 4B). Similar results were obtained from other comparison...
groups, such as between the SCI and EA groups, and 200 permutation tests demonstrated that these patterns have good reliability (Fig. 4C, Fig. S3A–F). The OPLS-DA model revealed 628 metabolites showing statistically significant differences and only two metabolites, including phenacetine and catechin overlapped among the four groups (Fig. 4D). Catechin abundance significantly decreased in the faeces of SCI rats but increased in the EA group. The differentially expressed metabolites among the groups are listed in Table S2.

**Integrated analysis reveals key intestinal microbiota associated with altered local phenol profiles in EA- and FMT-treated SCI rats**

To study the functional significance of the metabolic dysfunction induced by gut microbiota disturbances in SCI rats further, we performed pairwise correlation analysis to explore complex correlations between microbes and metabolites. The O2PLS results showed high inherent correlations between the datasets of metabolites and microbes (Fig. S4A–C). Pairwise correlation analysis was conducted to obtain a Circos plot; here, red and green lines indicate positive and negative correlations, respectively (Fig. S4D–F). For instance, between the SCI and EA groups, phenols were positively and negatively correlated with Bacteroidetes and Proteobacteria, respectively (Fig. S4E). This correlation was mainly attributed to phloretin (Fig. 5A). Between the SCI and FMT groups, phenols showed a more complex association with gut microbiota, and this correlation was mainly attributed to phenol 2, pyrocatechol and dopamine (Fig. 5B). For example, at the genus level, pyrocatechol was positively correlated with Pygmaiobacter, Papillibacter and Lactobacillus but negatively correlated with Bacteroides, Faecalibacterium, Anaerofustis, Subdoligranulum, Oscillospira and Helicobacter. Dopamine was positively correlated with Bacteroides, Elusimicrobium, Faecalibacterium, Fuscaltenibacter and Helicobacter but negatively correlated with Lactobacillus. Our results suggest that a potential link exists between gut microbiota alterations and metabolome changes in SCI rats due to EA and FMT treatment and that phenols may be involved in this link.
EA and FMT restore the expression of the 5-HT system in the intestine of SCI rats

To assess whether EA and FMT treatment modulates the function of the 5-HT system, we examined the expression of 5-HT-related molecules in the colon. The results of ELISA showed that SCI causes a significant decrease in the content of 5-HT in the colon and that EA and FMT treatment significantly restores this content (Fig. 6A). The expression of excitatory receptors, namely, 5-HT3AR and 5-HT4R, in the colon was consistent with the distribution trend of 5-HT content in each group (Fig. 6B and C). IHC confirmed the distribution of 5-HT3AR and 5-HT4R in response to EA and FMT treatment. As shown in Fig. 6D, in the sham group, the 5HT3AR and 5HT4R immunopositive cells were densely and continuously distributed in the location of the myenteric plexus in the colon, and the staining was brownish yellow; the 5HT3AR and 5HT4R were distributed in small amounts with yellowish in the SCI group; in the EA and FMT groups, 5HT3AR and 5HT4R immunopositive cells were densely and continuously distributed around the myenteric plexus in the colon, and the stain was brownish yellow. We also examined the expression of TPH1, TLR2, TLR4 and SERT. Compared with that in the sham group, the expression of TPH1 and SERT was downregulated in the SCI group; the expression of TLR2 and TLR4 was not significantly altered between these groups (Fig. 6E). EA and FMT treatment significantly reversed the SCI-induced downregulation of TPH1 and significantly downregulated the expression of TLR2, TLR4 and SERT. These results collectively imply that EA and gut microbes could regulate the 5-HT system in SCI rats via modulating 5-HT-related molecules.
By 16S rDNA sequencing, we found that the composition and evenness of the faecal flora of rats are significantly changed after SCI. At the phylum level, the abundance of Proteobacteria presented a significant increase in the SCI group but significantly decreased after EA and FMT treatment. Under normal circumstances, Proteobacteria occupies only a small proportion of the intestinal flora, and its expansion can be used as a criterion for the identification of potential microbial disorders and diseases (Shin et al., 2015). Abnormalities in the intestinal immune system can cause short-term expansion of the communities dominated by Proteobacteria and lead to intestinal inflammation and sensorimotor dysfunction (Maharshak et al., 2013). The expansion of Proteobacteria is generally observed in the intestines of children suffering from malnutrition, likely because nutritional metabolism disorders affect the balance of the flora (Subramanian et al., 2014). Therefore, Proteobacteria expands into the dominant microbiota community on account of its strong adaptability in SCI rats to aggravate the progression of the disease. However, administration of FMT, which is derived from EA rats, inhibited the expansion of Proteobacteria. Kigerl et al. (2016) supported this effect of FMT and demonstrated that probiotics administration could reverse microbiome dysbiosis, thereby promoting functional recovery in SCI mice. We noted some inconsistent results between the EA and FMT groups. Similar to findings on the Shannon index, differences between the sham and FMT groups, as well as between the EA and FMT groups, were observed (Fig. 2D). This finding may be attributed to the ability of FMT to alter the rat microbial community. Moreover, some microbes and metabolites existing in faeces may not be regulated by EA but exert some effect on the intestinal microbial composition resulting from the SCI. We recommend further explorations of these EA-induced microbes in follow-up studies.

At the class level, SCI induced significant increases in the relative abundance of Gammaproteobacteria in Proteobacteria, Erysipelotrichia and Clostridia in Firmicutes. The increase of these bacteria is associated with a variety of diseases, such as Crohn’s disease (Laffin et al., 2018) and infectious intestinal inflammation (Zeng et al., 2017). FMT mainly affects the colonization of Clostridia. Clostridia can be used as biological materials capable of ethanol (Demain et al., 2005), butanol (Lee et al., 2008), and other toxic substances. Bartlett et al. (1978) confirmed that Clostridia could produce cytotoxic substances leading to pseudomembranous colitis. Therefore, the protection afforded by FMT to SCI rats may be related to...
its ability to prevent *Clostridia* colonization to reduce the production of toxic substances. Although the treatment effects (e.g., on behaviour and morphology) of the FMT group were similar to those of the EA group, differences in the distribution of some bacteria, such as *Clostridia*, were also observed. The reason may be that the regulation of EA on rats is systemic and demonstrates the overall effect, and the remodelling of intestinal microbiome is just one of the important manners but not only one; while intestinal microbiome remodelling may be the only direct approach to FMT treatment.

In the process of EA amelioration SCI through microbiota, the communication between microbiota and recovery from SCI is important, it could be dependent on various routes, including the immune system, tryptophan metabolism system, and gut microbial metabolites, which may involve microbial metabolites such as metabolite catechin (Schroeder and Backhed, 2016; Sharon et al., 2016). Catechins are a class of bioactive polyphenols that are abundant in the human diet; these substances can induce weight loss and may be related to energy metabolism (Luo et al., 2018). Catechins are commonly detected in the microbial metabolites of the intestinal flora of rats and humans (Aura et al., 2008; Goodrich and Neilson, 2014). Catechins are believed to have antioxidant, antiapoptotic and anti-inflammatory properties and confer neuroprotection to the neural injury model (Khalatbary and Khademi, 2020). For example, epigallocatechin-3-gallate alters inflammatory cytokine levels to enhance neuroregeneration in SCI rats (Machova Urdzikova et al., 2017). Kutschera et al. (2011) demonstrated that *Clostridium orbiscindens* and *Eggerthella lenta* isolated from the human intestine are involved in the conversion of catechins. In our study, *Clostridia* was also identified to be differentially enriched in each group. Integrated analysis of microbiomes and metabolomes demonstrated that pyrocatechol, a precursor of catechins, is significantly associated with a variety of microbiota, including *Anaerofustis*, *Faecalibacterium* and *Subdoligranulum* in *Clostridia*. Thus, catechins may present a mechanism through which EA treatment improves SCI by stimulating alterations in microbial metabolites.

As an important mediator of intestinal function and SCI, the anabolic secretion of 5-HT is influenced by the intestinal flora and their metabolites. Previous studies found that the intestinal flora and their metabolites, particularly short-chain fatty acids, could stimulate 5-HT production through TPH1 in enterochromaffin cells (Reigstad et al., 2015). Yogesh et al. observed gut

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**Fig. 6.** EA and FMT restores the expression of the 5-HT system in the intestine of SCI rats.
A. Contents of 5-HT in the colon detected by ELISA (*n* = 10).
B. mRNA expression of 5-HT3AR in the colon detected by qRT-PCR (*n* = 5).
C. mRNA expression of 5-HT4R in the colon detected by qRT-PCR (*n* = 5).
D. Distribution and protein expression of 5-HT3AR and 5-HT4R in the colon detected by IHC (magnification, ×200, *n* = 5).
E. mRNA expression of TPH1, TLR2, TLR4 and SERT in the colon detected by qRT-PCR.
microbiota-driven changes due to 5-HT3R expression regulation through acetate production (Bhattarai et al., 2017). In mice that received FMT from patients with constipation, intestinal dysbiosis (including Clostridia) was accompanied by a significant upregulation of SERT expression and a decrease in 5-HT in colon, both of which contribute to the development of chronic constipation (Cao et al., 2017). Collectively, intestinal microbiota and its metabolites exert regulatory effects on the 5-HT system. In the present study, EA or FMT treatment (from EA-treated rats) could improve intestinal functions and restore 5-HT expression in SCI rats, thus suggesting that the 5-HT system is regulated by EA and FMT during the EA treatment of SCI. However, whether the regulation of 5-HT is mediated by the microbiota or the synergistic effects of microbiota and metabolites requires further study.

Earlier in the discussion, we suggested that the microbiota (such as Clostridia) and metabolites (such as catechin) play important roles in the improvement of the SCI process by EA and FMT; this process may be mediated by the 5-HT system. Study reported that gut microbiota of Clostridium ramosum (class Clostridia) could facilitate 5-HT secretion and promote intestinal lipid absorption (Mandic et al., 2019). Clostridium butyricum (class Clostridia) could also upregulate 5-HT expression. Enteropathogenic Escherichia coli (class Gammaproteobacteria) infection prevents proper intestinal 5-HT function and expression (Esmaili et al., 2009). Thus, the 5-HT system may be regulated by microbiota, which is consistent with our finding that FMT significantly restores the expression of 5HT3AR and 5HT4R in SCI rats. Moreover, the antinociceptive activity of (−) epicatechin requires the participation of the 5-HT system (Lopes Lda et al., 2012), as does the antinociceptive effect of (−)-epicatechin (Quinonez-Bastidas et al., 2018). Thus, the 5-HT system can be regulated by catechin isoforms. This evidence has led us to infer that EA promotes the recovery of intestinal function and the treatment of SCI by regulating intestinal flora and metabolites and communicating with the colon 5-HT system.

Given the fact that EA stimulation and FMT treatment improved bowel movement after SCI, we suspect that EA and FMT together may show synergistic effects and even greater benefits. FMT suspensions can be administered directly by gavage in animal studies, but the same approach is unacceptable in humans; thus, studies of this nature in humans are limited. Future work should explore the synergistic effects of EA and FMT in the human body and identify beneficial flora.

In summary, EA and FMT treatment promoted intestinal transmission functional recovery, significantly altered the intestinal microbiota composition, including Proteobacteria, Clostridia, and changed the faecal metabolic profile such as catechin, of SCI rats. EA and FMT treatment also restored the expression of the 5-HT system in the colon of SCI rat. Our results shed more light on the physiological and pathologic basis of SCI from the perspective of the microbiota–gut axis mediated by 5-HT. These findings offer new insights into the role of gut flora disorders and the consequent metabolite changes in SCI development.

**Experimental procedures**

Details of the experimental procedures are described in the Appendix. S1.

**Experimental in vivo paradigm**

All animal studies were approved by the Ethics Committee of Hainan Medical University, and efforts were made to minimize the number and pain of experimental animals. The license number of rats is [SCXK (Su) 2016-0010]. Female Sprague Dawley® (SD®) rat aged 2–3 months (Changzhou Cavens Laboratory Animal Co. Ltd., Jiangsu, China) with average initial weight of 200 ± 20 g were adaptively raised under a 12 h/12 h circadian rhythm at a room temperature (25°C), and had free access to food and water. Prior to the start of the experiment, the improved Basso–Beattie–Bresnahan (BBB; (Basso et al., 1995) exercise performance score of all rats was 21 points. After 1 week of adaptive feeding, the rats were randomly divided into four groups (i.e., the sham, SCI, EA, FMT groups), and the rats after the modelling group were raised individually. During the experimental intervention, no restriction on eating and drinking was implemented. All rats were intraperitoneally injected with 3% pentobarbital sodium (50 mg kg⁻¹ of body weight), anaesthetized, and shaved to expose the skin from approximately T10 to T13. The back surface of rats in the sham group (n = 30) was cut open, and the superficial fascia was separated bluntly with haemostatic forceps. The spinous process and lamina at T10–T13 were exposed to the air for 5 min and then sutured directly.

The rats in the SCI model group (n = 30) were suffered model construction by the free-weight dropping and knocking method, as described previously (Thomas et al., 1999). In brief, a T11–T12 laminectomy was performed to expose spinal cord, and then severe SCI at T11–T12 section was produced by striking the exposed spinal cord using a 10 g weight falling from 60 mm with a NYU Impactor I device (NYU Impactor I, W.M. Keck Center, USA). After compression and haemostasis, the muscle and dermal layers were sutured successively. The rats in the EA group (n = 30) underwent SCI model surgery followed by EA treatment. These rats were
subjected to EA on the second day after surgery. Treatment was conducted once a day for 14 consecutive days. In the experiment, bilateral Zusanli points were stimulated after conventional iodophor disinfection and then punctured to a depth of 5 mm with a disposable acupuncture needle. The needle was connected to the EA instrument and dilatational wave was 2Hz/15Hz, current intensity was 1–2 mA, with the needle handle was slightly vibrated, and the rats did not hiss as an appropriate degree of EA. The rats in the FMT group (n = 30) underwent FMT following SCI. From the second day after surgery, the fresh feces excreted by rats in the EA group was collected every day to prepare a 10% faecal flora suspension, which was given to rats in the FMT group by gavage at a dose of 1 ml 100 g⁻¹ body weight, once a day for 14 consecutive days.

For postoperative care, all rats were given intraperitoneal injections of 5000 U kg⁻¹ gentamicin for anti-infection on the day after surgery, once a day for 16 consecutive days. The lower abdomen, perineum, and hind limbs of the modelled rats were cleaned daily, and the hind limbs were passively moved. Assisted urination: The rats were held upright and the bladder was gently rubbed from top to bottom according to the Crede technique once every 12 h for urine excretion.

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Conflict of interests

The authors declare that there are no conflicts of interest.

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**Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Fig. S1.** EA and FMT treatment alters the dysbiosis of the gut microbiome in SCI rats (n = 10). A. Venn diagram of the number of OTUs in each group. B. Diversity index rarefaction curve of species diversity. C. Beta diversity of each group according to the results of the weighted-Wilcoxon test on microbial communities. D. Average relative abundance of microbial communities at the class level. E. Average relative abundance of microbial communities at the genus level.

**Fig. S2.** Differences in gut microbial communities among the four groups at five levels based on the Metastat method. Abundance distributions of different species among the four groups at the class (A), order (B), order (C), family (D), genus (E) and species (F) levels. The Metastat method was used to perform hypothesis testing on the species abundance data between groups to obtain p values, which were corrected to obtain q values. * indicates q < 0.05, ** indicates q < 0.01.
Fig. S3. Different metabolites among groups. (A) OPLS-DA score plots in faecal samples between the EA and SCI groups, between the FMT and SCI groups (B), and between the FMT and EA groups (C). (D) Results of permutation tests between the FMT and EA groups, between the FMT and SCI groups (E) and between the EA and SCI groups (F). Data were collected in positive-ion mode. Ellipses in the PCA and OPLS-DA score plots indicate 95% confidence intervals.

Fig. S4. O2PLS results and correlations between the datasets of metabolites and microbes. (A) Correlation of metabolites and microbes between the sham and SCI groups, between the sham and SCI groups (B) and between the SCI and FMT groups (C). Circos plots of the results of pairwise correlation analysis between the sham and SCI groups (D), between the EA and SCI groups (E) and between the FMT and SCI groups (F). Red and green lines indicate positive and negative correlations, respectively.

Table S1. Primers information used in this study.

Table S2. The differentially expressed metabolites between four groups.

Appendix S1. Experimental procedures.