

ROLE OF MICROGLIA IN THE SPINAL CORD IN COLON-TO-BLADDER NEURAL CROSSTALK IN A RAT MODEL OF COLITIS

Hypothesis / aims of study

Chronic pelvic pain syndrome (CPPS) including bladder pain syndrome/interstitial cystitis (BPS/IC) and irritable bowel syndrome (IBS) is defined as a disease entity with painful symptoms in the pelvic region and presents a major challenge to patients and health care providers. Previous epidemiological studies demonstrated that symptoms of BPS/IC and IBS are often overlapped [1]. In order to explain this complex pathology, pelvic organ “cross sensitization” has been proposed to play a role in the clinically overlapping symptoms of CPPS. Also, in various animal models, microglia in the spinal cord is shown to be involved in the central sensitization process underlying chronic pain conditions [2]. We therefore investigated whether microglia in the spinal cord contributes to the colon-to-bladder neural crosstalk to induce bladder overactivity and pain behaviour in a rat model of colitis.

Study design, materials and methods

Adult female SD rats were divided into 3 groups: A) control group, B) colitis group, and C) colitis + minocycline group. 50% trinitrobenzene sulfonic acid was administered into the distal colon through a catheter inserted via the anus in the groups B and C to induce experimental colitis, whereas the group A was administered vehicle. A PE10 catheter was inserted into the L6/S1 intrathecal space on the same day. Minocycline (200mg/day), a microglia inhibitor, was continuously infused through the intrathecal catheter using an osmotic pump in the group C, whereas the groups A and B were given vehicle. Following investigations were performed on day 7.

- (1) Continuous cystometry (CMG) was performed in an awake condition.
- (2) Nociceptive behaviour induced by intravesical instillation of resiniferatoxin (a TRPV1 agonist; 3 μ M for 30 min) such as licking behaviour (lower abdominal licking) and freezing behaviour (motionless head-turning towards lower abdomen) was observed.
- (3) Hematoxyline-eosine staining was performed for bladder and distal colon sections.
- (4) Immunofluorescence staining for CD 11b, a microglia marker, was performed for L6 spinal cord sections.
- (5) RT-PCR was performed for the L6 spinal cord tissue to investigate gene expressions such as IL-1 β and CCL3.
- (6) Toluidine blue staining that detects mast cell infiltration and activation was performed for the bladder.

Results

- (1) In CMG, the group B (n = 6) showed significantly ($p < 0.01$) shorter intercontraction intervals (ICI) than the group A (n = 7). The group C (n = 8) showed significantly ($p < 0.01$) longer ICI than the group B (Fig. 1).
- (2) There were no significant differences in licking events among 3 groups. However, the number of freezing events was significantly ($p < 0.01$) greater in the group B (n = 6) than in the group A (n = 6). The group C (n = 6) showed the significantly ($p < 0.05$) smaller number of freezing events than the group B (Fig. 2).
- (3) HE staining showed the infiltration of the inflammatory cells and submucosal bleeding in the distal colon of the group B and C compared with the group A. On the other hand, there were no such pathological findings in the bladder of any of three groups.
- (4) Immunohistochemistry for CD 11b in the L6 spinal cord demonstrated that the number of CD 11b positive cells was significantly different among 3 groups with the following order; group B (n = 7) > group C (n = 8) > group A (n = 7) (Fig. 3).
- (5) The mRNA expressions of IL-1 β and CCL3 in the L6 spinal cord were significantly increased in the group B (n = 7) compared with the group A (n = 7) ($p = 0.003$ and $p = 0.02$). However, the group C (n = 7) showed the significantly lower mRNA expressions of these molecules than the group B ($p = 0.01$ and $p = 0.01$) (Fig. 4).
- (6) In toluidine blue staining, the group B (n = 5) showed the significant larger number of total mast cells as well as degranulated ones in the bladder than the group A (n = 5) (68 ± 5 and 34 ± 2 vs. 27 ± 3 and 6 ± 1 , $p < 0.001$ and $p < 0.001$, respectively). The group C (n = 5) showed the significantly ($p < 0.05$) smaller number of total mast cells as well as degranulated ones in the bladder than the group B (29 ± 1 and 5 ± 1 , $p < 0.001$ and $p < 0.001$, respectively).

Fig. 1

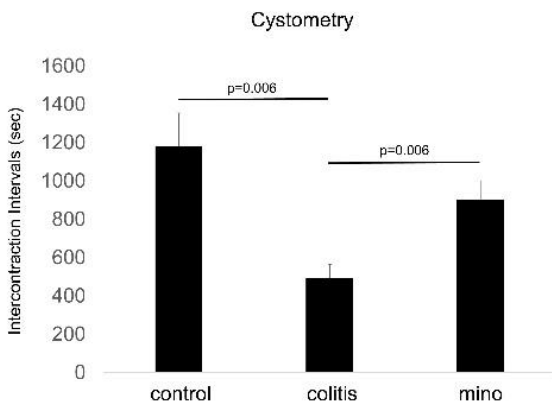


Fig. 2

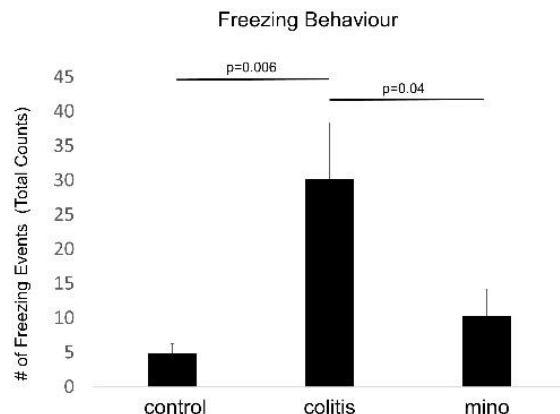


Fig. 3

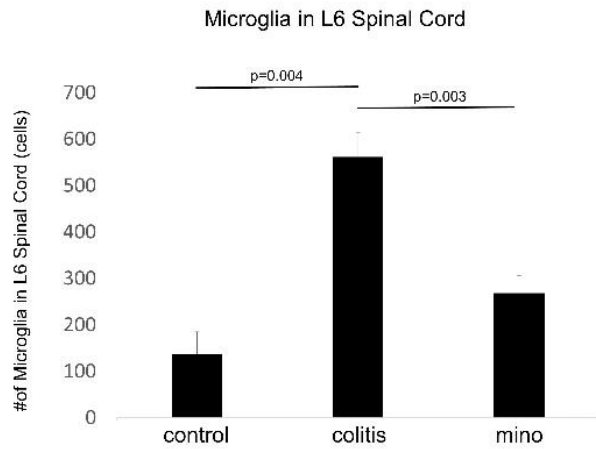
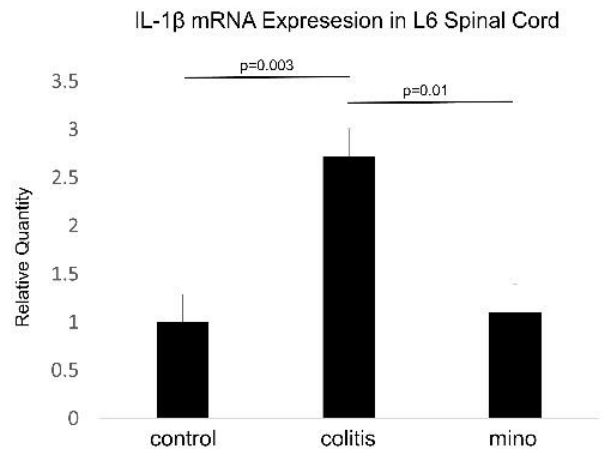


Fig 4



Interpretation of results

These results indicate that; (1) administration of tri-nitrobenzene sulfonic acid into the distal colon elicits frequent urination shown by reduced ICI, and enhanced bladder pain sensitivity shown by increased freezing behaviour, (2) colitis-induced bladder overactivity and enhanced bladder pain sensitivity are associated with increases in an infiltration of microglia and chemical mediators in the L6 spinal cord as well as an increase in infiltrated mast cells in the bladder and (3) intrathecal treatment of minocycline, which is known as a microglia inhibitor, results in the improvement of bladder pain behaviour and frequent urination induced by experimental colitis.

Concluding message

Microglia in the spinal cord is likely to play an important role in colitis-induced bladder overactivity and enhanced bladder pain sensitivity in colitis rats. Therefore, spinal microglia activation might be a potential therapeutic target for overlapped pelvic pain disorders such as BPS/IC and IBS.

References

1. Nickel JC, Tripp DA, Pontari M, Moldwin R, Mayer R, Carr LK, Doggweiler R, Yang CC, Mishra N, Nordling J. Interstitial cystitis/painful bladder syndrome and associated medical conditions with an emphasis on irritable bowel syndrome, fibromyalgia and chronic fatigue syndrome. *J Urol.* 2010; 184: 1358-63.
2. Old EA, Clark AK, Malcangio M.: The role of glia in the spinal cord in neuropathic and inflammatory pain. *Handb Exp Pharmacol.* 2015; 227: 145-70

Disclosures

Funding: DOD W81XWH-12-1-0565 **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Nagoya University & University of Pittsburgh Institutional Animal Care and Use Committees