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SHORT COMMUNICATION

Acute postoperative pain exacerbates neuroinflammation and related delirium-like cognitive dysfunction in rats

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Abstract

The acute neuroinflammatory response to surgery may play a key pathogenic role in postoperative delirium (POD). Here, we investigated the contribution of acute postoperative pain to neuroinflammation and related delirium-like behaviors after surgery in adult and aged rats. Animals were assigned into four groups: control, abdominal surgery, surgery with analgesia using local ropivacaine, and surgery with analgesia using systemic morphine. Pain was assessed by the Rat Grimace Scale (RGS). Trace and context memory retention was evaluated following trace fear conditioning during the first 2 days after surgery. Pro-inflammatory cytokines in medial prefrontal cortex and hippocampus were measured by enzyme-linked immunosorbent assay. In both age groups, the RGS increased significantly from baseline until 6 h after surgery. The postoperative analgesia with either local or systemic regimens comparably alleviated the RGS increase in adult and aged animals. The two analgesic regimens attenuated the surgery-induced trace and context memory deficits, as well as cytokines overproduction in both medial prefrontal cortex and hippocampus. No age-related differences were found in the neuro-cognitive effectiveness of postoperative analgesia. Our experimental findings provide proof-of-concept for adequate postoperative pain management as one of the main preventive strategies of POD.

Keywords Postoperative pain · Delirium · Neuroinflammation

Short communication

Postoperative delirium (POD) is a common surgery-related complication especially in the elderly patients [1–3]. The consequences of POD can be serious, including associations with long-term cognitive decline and increased mortality. To date, no established pharmacological prevention or treatment for POD has been available. The latest clinical practice guidelines suggest that a multicomponent preventive intervention minimizing known risk factors may be the best approach to reduce the incidence of POD [2, 3]. Among them, acute postoperative pain is widely accepted to be one of the most significant modifiable contributors. Nevertheless, preclinical direct evidence regarding the association between acute postoperative pain and POD is entirely lacking.

Although the exact pathogenesis of POD remains underdetermined, previous studies imply a critical role of neuroinflammation characterized by overproduction of pro-inflammatory cytokines within the brain [4, 5]. More recently, we reported in rats that abdominal surgery induced acute widespread neuroinflammation and related POD-like state in the early postoperative period [6]. This neuroinflammation may transit from acute to chronic in an age- and hippocampal-specific manner, resulting in the development of sustained cognitive dysfunction. We have further demonstrated that acute postoperative pain contributes to the development of cognitive dysfunction during the late period after surgery in aged rats [7]. However, the effects of postoperative pain on acute neuroinflammation, as well as its age dependency

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on related delirious state, remain unknown. In this study, therefore, we investigated the contribution of postoperative pain to POD-associated acute neuroinflammatory responses in adult and aged rats.

All experiments were approved by the Institutional Animal Care and Use Committee of Kochi Medical School (Approval number: H-00121). Male adult (2–4 months) and aged (20–24 months) Wistar rats were housed with standard 12-h cycle lighting with ad libitum access to food. The rats of each age were randomly allocated to one of four groups ($n = 8$ rats/group): (1) anesthesia without surgery (control group); (2) anesthesia with surgery; (3) anesthesia with surgery plus single-dose surgical wound infiltration with 0.2% ropivacaine after surgery; and (4) anesthesia with surgery plus a single subcutaneous dose administration with 0.8 mg/kg morphine after surgery. 1 week before the surgical procedure, all randomized rats were subjected to a standard open field test.

Abdominal surgery was performed as previously described [6–8]. Briefly, 1.5–2.0% isoflurane was maintained through a nose cone during the procedure. Following a 2 cm midline laparotomy, the small intestine was exteriorized, and manipulated with fingers for 3 min. The surgery duration was fixed at 10 min. The control rats were only anesthetized for the same time period. The hemodynamic parameters during anesthesia were non-invasively measured. Postoperative pain was assessed by the Rat Grimace Scale (RGS) from 0 (no pain) to 2 (severe pain) according to a previous report [9]. The RGS was recorded 10 min before surgery, at 2, 4, 6, 8, 12, and 24 after inhalation period.

Trace and context memory retention after the trace fear conditioning was conducted to assess medial prefrontal cortex (mPFC)-dependent attention and hippocampus-dependent memory function, respectively, as our previous study [6]. Briefly, on the first day after surgery, each rat was placed in the conditioning chamber and received one training session of 8 trials of trace fear conditioning, consisting of a 10-s, 4 kHz, 75 dB tone conditioned stimulus (CS) and a 0.7-s, 0.8 mA foot shock unconditioned stimulus (US) separated by an empty 30-s trace interval. 24 h after the training session, the trace and context memory retention tests were performed. During the trace memory retention testing, the animal was placed in a novel context consisting of an observation chamber for a 2-min baseline (pre-CS) period. Then, a 10-s CS tone without the foot shock was given, followed by a 4-min post-CS period. Total seconds of freezing during the post-CS period were measured, and shown as a percentage of freezing time in 4 min (% freezing). Freezing behavior is defined as the absence of all movement, excluding movement caused by respiration. For the context memory retention testing, the freezing behavior was recorded during exposition to the original conditioning chamber for a 4-min period without the CS or US. After behavioral testing, the animals were decapitated. The hippocampus and mPFC were rapidly harvested and homogenized with a polytron homogenizer in ice-cold lysis buffer containing protease inhibitors. The levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were analyzed by the enzyme-linked immunosorbent assay.

The results were expressed as the mean ± standard deviation (SD). Differences between the study groups were compared with the Kruskal–Wallis test and differences between individual groups with the Wilcoxon–Mann–Whitney test with Bonferroni correction. Data were analyzed using the statistical software SPSS (version 11; SPSS Inc, Chicago, IL). $p < 0.05$ was considered statistically significant.

There were no significant differences in the parameters measured in the preoperative open field test (Supplementary data 1) and hemodynamics during anesthesia (Supplementary data 2) among experimental groups. Body weights were not significantly different from baseline (before surgery) to 2 days after surgery in all groups. In both adult and aged groups, the RGS increased significantly from baseline until 6 h after surgery. However, the two analgesic regimens comparably suppressed the postoperative RGS increase (Supplementary data 3). During the training session, no group or age difference was observed in the freezing responses (Supplementary data 4), indicating intact acquisition of fear memory in the conditioning. For trace memory retention, freezing behaviors were comparably short during the 2-min baseline (before CS) in all groups in both adult (Fig. 1a) and aged (Fig. 1b) groups. However, significant group effects were observed during the 30-s trace interval following the CS offset. Subsequent pairwise comparisons demonstrated that surgical rats froze significantly less than control rats, whereas aged surgical rats froze less than adult surgical rats ($p < 0.05$). Notably, either of the 2 analgesia regimen inhibited the laparotomy-induced trace memory deficit with similar potency within adult groups, as well as within aged groups. However, the age differences in trace memory were still observed, i.e., % freezing in either analgesia group of aged animals was significantly lower compared with the corresponding group of adult rats. For the context retention testing, pairwise comparisons indicated that surgical rats froze significantly shorter than control animals in both adult ($p < 0.05$) and aged ($p < 0.05$) groups. However, no significant differences in freezing levels were found between age groups. The surgery-induced context memory deficit was comparably attenuated by the 2 analgesia regimens in both age groups.

The average TNF-α levels in the hippocampus and mPFC of the surgical group were significantly greater than those of the control group in both adult (Fig. 2a, b) and aged (Fig. 2c, d) animals. However, the TNF-α levels of ropivacaine and morphine group were comparably lower than those in surgical group ($p < 0.05$ in each pairwise comparison).

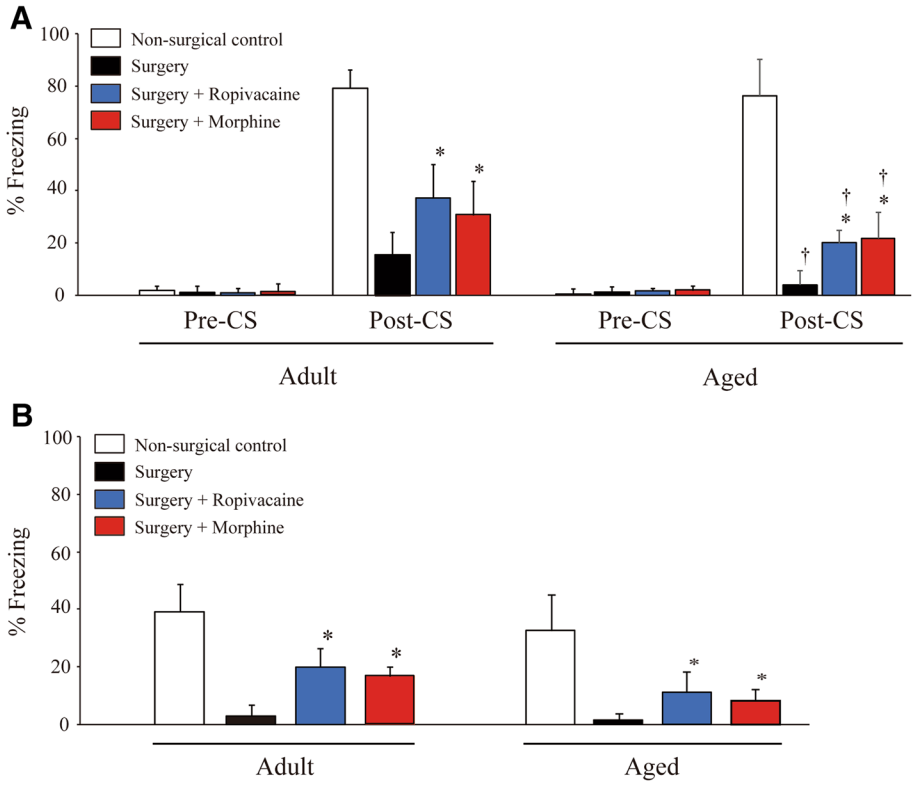

Fig. 1 Behavioral response to trace fear conditioning during the testing session in adult and aged animals at 2 days after surgery. **a** Freezing behavior during trace memory retention test before the tone (Pre-CS) and after (Post-CS). **b** Freezing behavior during context memory retention test. The four study groups were as indicated in materials and methods ($n=8$ in each group). Each vertical bar represents the mean ± SD. $^{*}p<0.05$ vs. surgery group within each age group. $^{\dagger}p<0.05$ vs. corresponding group in adult rats

Age-dependent group differences in TNF-α production were not observed ($p>0.05$ in each pairwise comparison). Regarding IL-1β levels, the statistical analysis revealed similar main group effects with those observed for TNF-α in both age groups.

Our results demonstrated that postoperative analgesia with either local ropivacaine infiltration or systemic morphine could ameliorate acute neuroinflammation and related delirious-like cognitive disorders. Interestingly, despite the entirely different analgesic mechanisms, both analgesic regimens showed comparable effectiveness. Furthermore, preliminary data further indicated that non-surgical animals that received analgesic regimens failed to show significant effects in trace or context memory, as well as brain cytokine levels (Supplementary data 5). These observations suggest that the anti-neuroinflammatory responses observed in the analgesic groups were mediated via pain-relieving effects, rather than through any direct effects on cognitive function.

The peripheral inflammatory signals are known to be transferred into the brain via several pathways [10]. In particular, the afferent vagus nerve is reported as an important neuronal transmitting route [11]. Our findings further imply that afferent pain signaling may also be involved in the peripheral-to-brain immune communication. In addition, recent evidence indicates that stress exposure exaggerates the neuroinflammatory responses to a subsequent peripheral immune challenge via the neuroendocrine pathway [12]. Therefore, another possibility is that the stress response to acute pain may trigger the development of neuroinflammation. In either cases, adequate postoperative acute pain management may be important to optimize the neuroinflammatory response to surgery, and thus minimize the incidence of POD.

Despite similar brain cytokine levels, surgery-induced impairment of trace fear memory retention was more pronounced in aged than adult animal (Fig. 1a). These results complement the clinical observation that the elderly patients are more vulnerable to the development of POD. However, analgesic regiments failed to diminish this age-related behavioral susceptibility. This phenomenon indicated that intrinsic brain aging conditioning, but not excessed peripheral stimuli, might be involved in the predisposition to POD in the elderly.

There are few limitations that should be considered. First, the present results along with our previous findings [7] imply that adequate management of acute postoperative pain may prevent both early and late cognitive decline after surgery. However, the effects of postoperative pain on the chronic neuroinflammation during the late period after surgery have not yet been directly examined. Second, intact acquisition of fear memory was observed during the training session in all experimental groups (Supplementary data 4). Nevertheless, we cannot rule out the possibility that acute pain could modify the sensitivity to CS and/or US, interfering with the behavioral testing. Third, the invasive intensity of our surgical model may not exactly

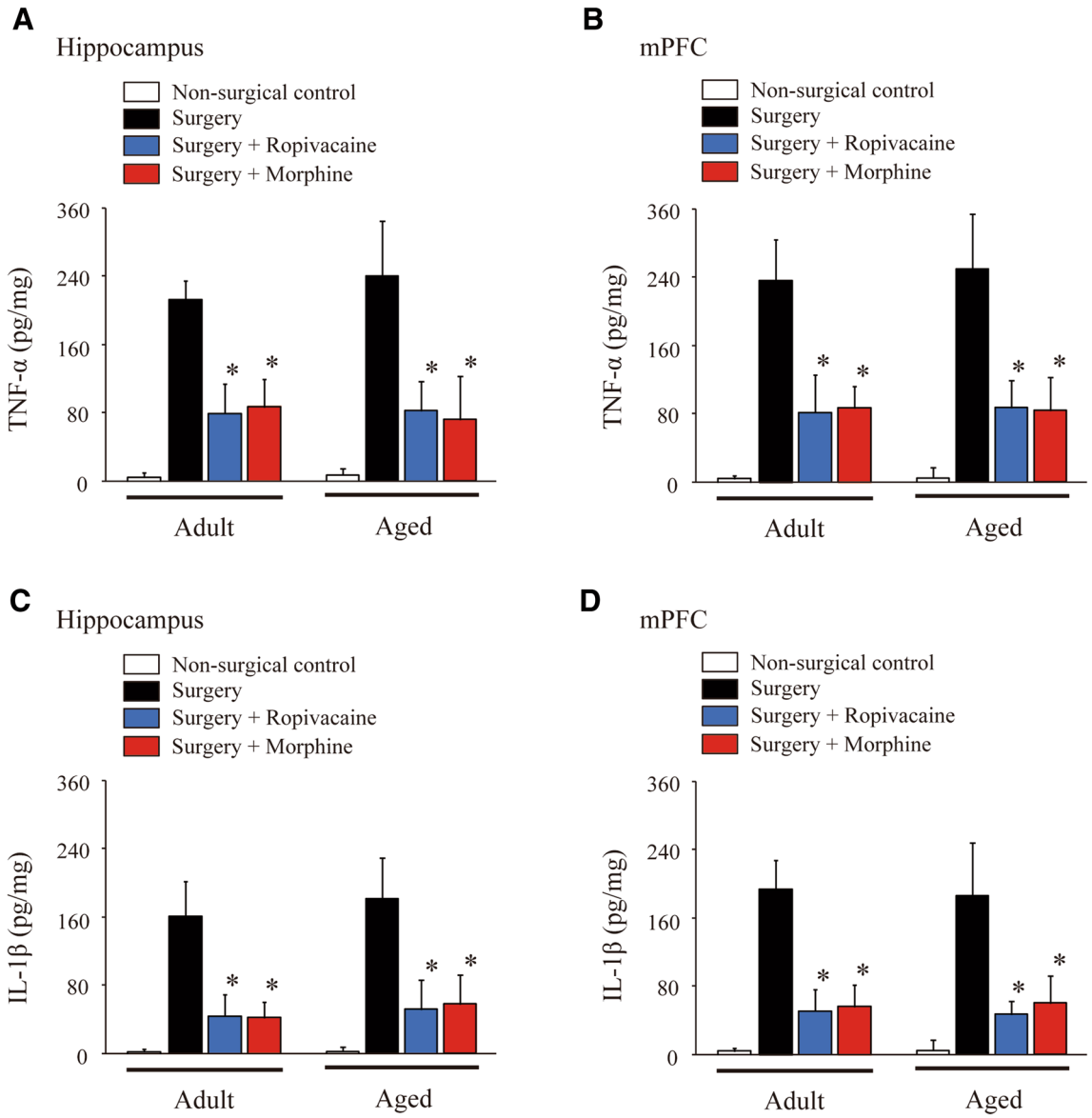

Fig. 2 Pro-inflammatory cytokine levels in the brain after surgery in adult and aged rats. Tumor necrosis factor (TNF)-α levels in the hippocampus (**a**) and medial prefrontal cortex (mPFC, **b**), as well as interleukin-1β (IL-1β) levels in the hippocampus (**c**) and mPFC (**d**), were measured by enzyme-linked immunosorbent assay. Each vertical bar represents the mean ± SD ($n = 8$ in each group). *$p < 0.05$ vs. surgery group within each age group

simulate the complex clinical situation. This may explain why each analgesia regimen could almost completely reduce the postoperative pain. Therefore, future studies are needed to translate our preclinical animal findings to humans.

In conclusion, the results of our proof-of-concept study indicated that acute postoperative pain may contribute to the development of neuroinflammation and related delirious-like behavior after surgery. Our findings support that postoperative pain management may be one of the most important modifiable factors for prevention of POD.

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References

1. Deiner S, Silverstein JH. Postoperative delirium and cognitive dysfunction. Br J Anaesth. 2009;103:i41–6.

2. American Geriatrics Society Expert Panel on Postoperative Delirium in Older Adults. Postoperative delirium in older adults: best practice statement from the American Geriatrics Society. J Am Coll Surg. 2015;220:136–48.
3. Aldecoa C, Bettelli G, Bilotta F, Sanders RD, Audisio R, Borozdina A, Cherubini A, Jones C, Kehlet H, MacLullich A, Radtke F, Riese F, Slooter AJ, Veyckemans F, Kramer S, Neuner B, Weiss B, Spies CD. European Society of Anaesthesiology evidence-based and consensus-based guideline on postoperative delirium. Eur J Anaesthesiol. 2017;34:192–214.
4. Liu X, Yu Y, Zhu S. Inflammatory markers in postoperative delirium (POD) and cognitive dysfunction (POCD): a meta-analysis of observational studies. PLoS One. 2018;13:e0195659.
5. Munster BC, Aronica E, Zwinderman AH, Eikelenboom P, Cunningham C, Rooij SE. Neuroinflammation in delirium: a postmortem case-control study. Rejuvenation Res. 2011;14:615–22.
6. Kawano T, Yamanaka D, Aoyama B, Tateiwa H, Shigematsu-Locatelli M, Nishigaki A, Iwata H, Locatelli FM, Yokoyama M. Involvement of acute neuroinflammation in postoperative delirium-like cognitive deficits in rats. J Anesth. 2018;32:506–17.
7. Chi H, Kawano T, Tamura T, Iwata H, Takahashi Y, Eguchi S, Yamazaki F, Kumagai N, Yokoyama M. Postoperative pain impairs subsequent performance on a spatial memory task via effects on *N*-methyl-D-aspartate receptor in aged rats. Life Sci. 2013;93:986–93.
8. Locatelli FM, Kawano T, Iwata H, Aoyama B, Eguchi S, Nishigaki A, Yamanaka D, Tateiwa H, Shigematsu-Locatelli M, Yokoyama M. Resveratrol-loaded nanoemulsion prevents cognitive decline after abdominal surgery in aged rats. J Pharmacol Sci. 2018;137:395–402.
9. Sotocinal SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JC, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, Mogil JS. The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol Pain. 2011;7:55.
10. Dantzer R, Konsman JP, Bluthé RM, Kelley KW. Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent? Auton Neurosci. 2000;85:60–5.
11. Chavan SS, Pavlov VA, Tracey KJ. Mechanisms and therapeutic relevance of neuro-immune communication. Immunity. 2017;46:927–42.
12. Calcia MA, Bonsall DR, Bloomfield PS, Selvaraj S, Barichello T, Howes OD. Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. Psychopharmacology. 2016;233:1637–50.