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Pregabalin can prevent, but not treat, cognitive dysfunction following abdominal surgery in aged rats

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ABSTRACT

Aims: The present study aimed to explore the preventive or therapeutic effect of peri-operative pregabalin treatment on the memory deficits and related hippocampal inflammation following surgery in aged rats.
Main methods: Aged rats underwent abdominal or sham surgery, and were then divided into 2 groups, either early or late pregabalin treatment. Fourteen days after surgery, the cognitive function was assessed using novel object recognition test, followed by measurement of hippocampal cytokines and voltage-dependent calcium channel α2δ subunit (CACNA2D1). The parabiotic experiments determined whether the humoral or neuronal pathway was involved in the neuroinflammation development following the abdominal surgery. The effects of pregabalin on LPS-induced cytokine release from hippocampal microglia were also evaluated.
Key findings: Early pregabalin treatment, which was administered pre-operatively and continued for 3 or 7 days after surgery, prevented memory deficits and decreased hippocampal pro-inflammatory cytokine levels. In contrast, no beneficial effects were observed when pregabalin was administered late in the post-operative period. The hippocampal levels of CACNA2D1 did not change under any experimental condition. The data from the cross-circulation (parabiosis) experiments indicated that abdominal surgery may induce neuroinflammation *via* a neural transmission pathway from the periphery to the brain. The *ex vivo* experiments further demonstrated that pregabalin had no effect on LPS-induced cytokines release from hippocampal microglia.
Significance: Our findings highlight reveal that peri-operative pregabalin treatment during the early post-operative period can prevent neuroinflammation and memory deficits after surgery. It is likely this occurs through a peripheral and central neuro-immune interaction rather than through direct anti-inflammatory effects.

1. Introduction

Post-operative cognitive dysfunction (POCD) is a common complication for geriatric surgical patients [1, 2]. Epidemiological studies demonstrate that POCD is not a transient phenomenon, but is associated with long-term disability and increased mortality [3]. Since no management strategy is currently available, it is imperative to develop specific methods for POCD prevention and management.

Although POCD pathogenesis involves various factors, accumulating evidence suggests that the inflammatory responses in the hippocampus have key roles in POCD development [4–6]. In particular, surgery-induced peripheral immune challenges can cause prolonged neuroinflammation, which is characterized by maladaptive microglial activation and the overproduction of pro-inflammatory cytokines, mainly interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) [7–9]. This process is exacerbated by aging and implicated in neurodegeneration, synaptic abnormalities, and apoptotic neuronal cell death [10]. All of these may be associated with cognitive impairments. Consistent with these findings, age-related microglial phenotype changes can contribute to POCD development in rodents [11]. Therefore, surgery-induced neuroinflammatory processes involving microglia may be a promising approach for understanding POCD pathogenesis.

Pregabalin was initially developed as an anticonvulsant for epilepsy, and subsequently demonstrated efficacy for neuropathic pain [12, 13]. Although it remains controversial, the principle pharmacological mechanism underlying pregabalin's effects is its ability to bind the α2δ subunit of voltage-gated calcium channels [12, 13]. The α2δ subunit is widely distributed in the central nervous system, including in the hippocampus [14, 15], and modulates excitatory neurotransmitters release [16–18]. Based on these characteristics, we hypothesis that pregabalin could regulate surgery-induced, microglia-mediated neuroinflammation and be effective for POCD.

Recently, we described an animal model of POCD, which was developed using abdominal surgery [11]. In this model, the level of cognitive

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decline positively correlated with age, as well as the degree of hippocampal microglia-mediated neuroinflammation. Using this model, we investigated whether peri-operative pregabalin administration could effectively prevent or treat recognition memory impairments and hippocampal neuroinflammation after abdominal surgery in aged rats. Furthermore, to explore the contribution of microglia in the hippocampus, we examined the effects of pregabalin on microglial responses to pro-inflammatory stimuli in *ex vivo* preparations.

2. Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Kochi Medical School. Male Wistar rats (24–25 months; 585–650 g) were used in this study.

2.1. Anesthesia and surgery

The rats were placed in an induction chamber, and anesthesia was induced with isoflurane in oxygen (induction: 3.0% at 2 l/min; maintenance: 1.5–2.0% at 0.5 l/min). The abdominal surgery was a 2 cm midline incision through the skin and abdominal muscles. During the laparotomy, the small intestine was exteriorized from the peritoneal cavity, covered with a moist gauze, and then manipulated with fingers for 3 min. The muscle and skin were then repaired separately with 5-0 Vicryl sutures. At the end of surgery, post-operative analgesia was induced by wound infiltration with 0.2% ropivacaine. In all experiments, the surgical duration was strictly standardized to 10 min, and body temperature was continuously monitored with a rectal probe and maintained at 37 °C with a heating lamp. During the isoflurane anesthesia, the mean arterial pressure was measured by tail-cuff plethysmography; the arterial oxygen saturation and pulse rate were measured noninvasively. The sham control animals were anesthetized and given analgesia in the same manner as the surgical rats, but did not receive the surgery. Our preliminary data (Supplementary Data 1) demonstrate that there is hippocampal neuroinflammation at Days 3 and 7, with a peak on Day 14 after surgery. However, these high cytokine levels were restored to almost normal values at Day 21. Therefore, we selected 14 days after surgery as an optimal assessment point of POCD in our model.

2.2. Pregabalin and time of administration

Sham control (non-surgical) or surgical rats were injected with pregabalin using 1 of 2 regimens: 1) early treatment, which was during the pre- and early post-operative period, or 2) late treatment, which was during the late post-operative period. The time course of each experimental group is schematically depicted in Fig. 1. Within each treatment group, the animals were administered either pregabalin or vehicle (administered in an equivalent volume) for an identical period of time. The control animals in the early and late treatment groups were administered vehicle alone (E-control and L-control, respectively). For the early treatment group, pregabalin (10 mg/kg) was administered intraperitoneally (*i.p.*) either 1 h prior to surgery alone (E: 0), 1 h prior to surgery and then every 6 h for 3 (E: 0–3) or 7 (E: 0–7) days. For the late treatment group, pregabalin (10 mg/kg) was injected during post-operative days 4–7 (L: 4–7) or 4–13 (L: 4–13). Each treatment group consisted of 12 animals, which was based on our previous study [11] and pilot data with a similar animal model.

Pregabalin (Sigma-Aldrich, St. Louis., MO) was diluted in the vehicle solution (0.9% phosphate buffered saline). The pregabalin dose was determined to be an effective analgesic dose in neuropathic rats [19].

2.3. Open field test

Rats were individually placed in a square open field (75 cm × 75 cm), which was a novel environment. The open field behavior was videotaped for 20 min using a camera that was placed above the arena. The videos were subsequently analyzed digitally using Noldus software (Noldus, The Netherlands).

2.4. Novel object recognition task

After the 14 day post-surgical recovery period, cognitive function was assessed using a novel object recognition test, which was previously described [11, 20]. Briefly, the plastic objects were similar in size, but different in color and shape. During the training session, 2 novel objects were symmetrically placed into the open field arena, which the subject animal was allowed to explore for 5 min. Object exploration was defined as the time spent sniffing the object when the rat's nose was in contact with the object and/or within 1 cm of the object. After a 1 h interval, the rat was returned to the experimental chamber, which contained 1 identical and 1 novel object, for the testing session. The rat was allowed to explore the objects for 5 min. All testing was conducted during the dark phase of the light/dark cycle in a dimly lit room and video-recorded. The videos were analyzed by an experimenter who was blind to the experimental group. In order to measure recognition memory, a preference index was calculated for the training and testing sessions. The preference index was defined as the ratio of time spent exploring an object during the training session, or the novel object during the testing session, over the total object exploration time.

After the behavior testing was completed, the hippocampus was rapidly dissected on ice, and homogenized with a polytron homogenizer (Kinematica Inc., Littau, Switzerland) in ice-cold lysis buffer containing a protease inhibitor cocktail (P8340, Sigma-Aldrich). The homogenates were centrifuged, and the supernatant was aliquoted and frozen at −80 °C until the ELISA analysis.

2.5. Parabiosis

The parabiosis surgery was performed under aseptic conditions, as described previously [21] with some modifications. Briefly, after isoflurane anesthesia, the corresponding lateral aspects of each weight-matched male rat were shaved, and a longitudinal skin incision from the shoulder to the knee joint was made on 1 side of each animal. The muscles were removed from the scapulae, and the bones were sutured together. The muscles around the scapulae and femurs were sutured together. A 6-0 nylon suture was used to approximate the dorsal and ventral skin. After surgery, the partners were housed as 1 pair per cage. To verify the cross-circulation between the parabiotic rats, 1 animal in the pair was injected intravenously with 350 μl of 0.25% Evan's Blue 4 weeks after the surgical union. Two hours after injection, the Evan's Blue concentrations were equalized between the injected and uninjected partners.

Six week after the parabiosis surgery, all parabiotic rat pairs were divided into 3 groups: 1) a control group (6 pairs), in which both rats were anesthetized and given analgesia; 2) a laparotomy group (7 pairs), in which 1 of the parabiotic partners received abdominal surgery; or 3) a pregabalin group (6 pairs), in which 1 of the partners was subjected to abdominal surgery, while both members were simultaneously administered early pregabalin treatment (E: 0–3). After a 14 day post-surgical recovery period, the hippocampi were harvested and analyzed with an ELISA for cytokine levels.

2.6. Acute isolation of hippocampal microglia

In order to examine the effects of the pregabalin regimens on the pro-inflammatory phenotype of hippocampal microglia, we performed another experiment using an identical surgical and pregabalin treatment protocol as shown in Fig. 1 (surgical group alone). At post-operative day 14, the microglia were acutely isolated from the hippocampus, which was previously described [11]. Briefly, the hippocampi were minced into pieces with a razor blade and were digested with 0.1%