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SHORT COMMUNICATION

Effects of ketoprofen for prevention of postoperative cognitive dysfunction in aged rats

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Abstract Postoperative cognitive dysfunction is a common geriatric complication that may be associated with increased mortality. Here, we investigated the effects of postoperative analgesia with ketoprofen on cognitive functions in aged animals and compared its effectiveness to morphine. Rats were randomly allocated to one of four groups: isoflurane anesthesia without surgery (group C), isoflurane anesthesia with laparotomy (group IL), and isoflurane anesthesia with laparotomy plus postoperative analgesia with ketoprofen or morphine. There was no difference in postoperative locomotor activity among groups. In group IL, postoperative pain levels assessed by the Rat Grimace Scale significantly increased until 8 h after surgery, which was similarly inhibited by both ketoprofen and morphine. Cognitive function was assessed using radial arm maze testing for 12 consecutive days from postoperative day 3. Results showed that the number of memory errors in group IL were significantly higher than those in goup C. However, both ketoprofen and morphine could attenuate the increase in memory errors following surgery to a similar degree. Conversely, ketoprofen showed no effect on cognitive function in the nonsurgical rats that did not experience pain. Our findings suggest that postoperative analgesia with ketoprofen can prevent the development of surgery-associated memory deficits via its pain-relieving effects.

Keywords Rehabilitation · Inflammation · Cognitive dysfunction · Aged rats

Postoperative cognitive dysfunction (POCD) is a mild but possibly long-lasting decline in cognitive function after anesthesia and surgery [1, 2]. Current literature has consistently shown that POCD is more frequent in elderly patients and is associated with long-term mortality [1–3]. Therefore, the treatment and prevention of POCD, which are of critical importance, are challenging because of the unclear causes. Recently, we demonstrated that postoperative acute pain could exacerbate memory deficits after laparotomy under isoflurane anesthesia in aged rats [4]. This result implies that effective pain management may be important for preventing the development of POCD in elderly patients. However, which analgesics are most appropriate remains to be determined.

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used as analgesics in the treatment of acute postoperative pain. NSAIDs attenuate inflammation by inhibition of the formation of prostaglandins via cyclooxygenase (COX) activity in both peripheral and central tissues [5, 6]. Although the precise mechanisms remain to be defined, recent studies suggest that an inflammatory response may

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be a key contributor to the development of POCD [7, 8]. Therefore, effective postoperative pain management with NSAIDs might potentially have beneficial effects in preventing the development of POCD. To test this hypothesis, we assessed the impact of NSAID ketoprofen-based analgesia on the development of memory deficits in aged rats after surgery and compared its benefits with those of opioid morphine-based analgesia.

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Kochi Medical School. Wister male rats aged 24–25 months were randomly allocated to the following four groups ($n = 8$ rats/group): isoflurane anesthesia without surgery (group C), isoflurane anesthesia with laparotomy (group IL), and isoflurane anesthesia with laparotomy plus a single subcutaneous administration with 40 mg/kg ketoprofen (group IL + ketoprofen) or 0.8 mg/kg morphine (group IL + morphine) after surgery. The 0.8 mg/kg dose of morphine was decided on after preliminary studies that showed dose-dependent (0.1, 0.4, 0.8, 1.2 mg/kg; $n = 4$–6, each dose) inhibition of RGS 4 h after laparotomy. The median antinociceptive dose of morphine was calculated as 0.42 mg/kg, and the dose of 0.8 mg/kg was high enough to almost completely inhibit the increase in RGS. The dose of ketoprofen (40 mg/kg) was also chosen based on preliminary dose–response studies (10, 20, 40, 60 mg/kg; $n = 4$–6, each dose); it provided the same degree of analgesia as the 0.8 mg/kg dose of morphine. Isoflurane was administered with 100 % oxygen at 1.0 minimal alveolar concentration (1.2 %) for aged rats. Laparotomy was performed by making a 1.0-cm midline longitudinal incision through the skin, the abdominal muscle, and the peritoneum. The muscle layers were repaired with 5-0 Vicryl sutures, and the skin was closed with tissue adhesive glue. During the 15-min inhalation period, pulse rate, arterial oxygen saturation, and mean arterial blood pressure were measured noninvasively. In a separate experiment, we tested whether ketoprofen showed direct effects on memory function by treating the rats in group C with either the analgesic or an identical volume of physiological saline ($n = 8$ rats/group). Postoperative pain was rated by the Rat Grimace Scale (RGS), developed by Sotocinal et al. [9], and recently validated in aged rats by our laboratory [10]. The overall RGS score was calculated by taking the average score (0–2) of all four RGS action units: orbital tightening, nose/cheek fluttering, ear position, and whisker

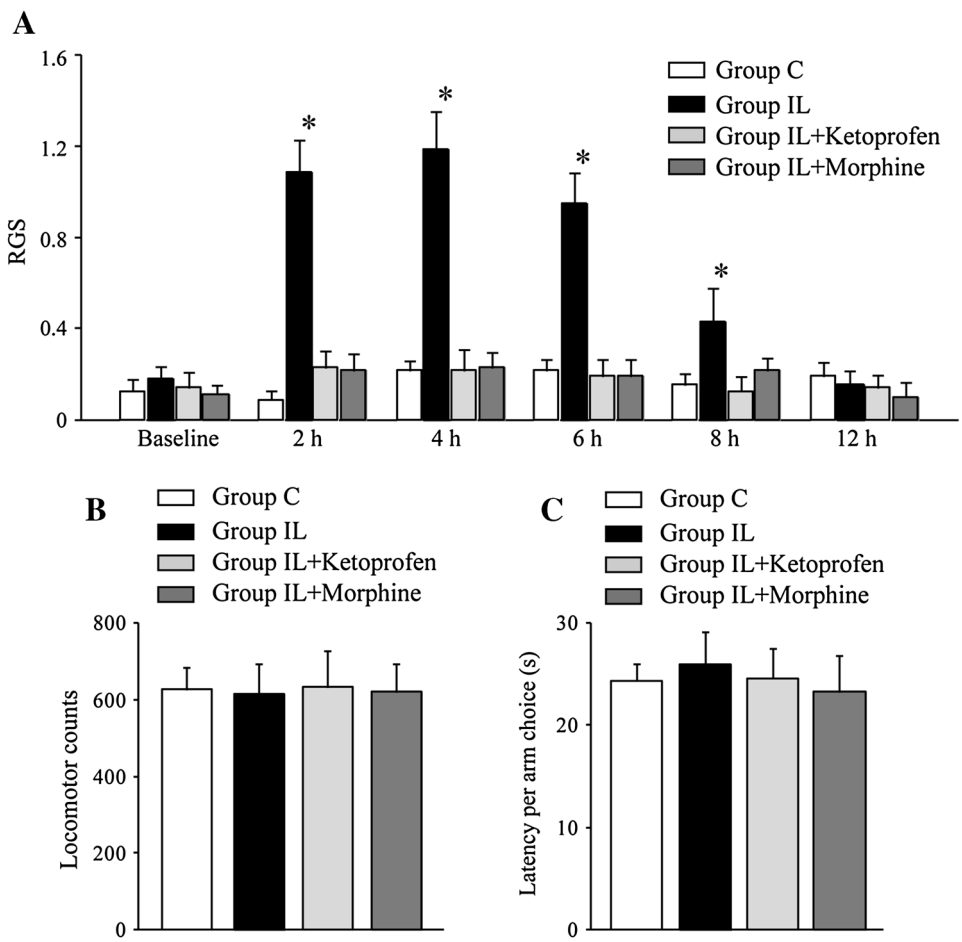

Fig. 1 Comparison of intensity of acute pain and physical activity between experimental groups. **a** Rat Grimace Scale (*RGS*) was scored at baseline and 2, 4, 6, 8, and 12 h after cessation of the inhalation period. $*p < 0.05$ vs. baseline. **b** Spontaneous locomotor activity was evaluated using the open-field test on postoperative day (POD) 3. Locomotor activity was measured by number of beam breaks. **c** The average latency per arm choice was calculated to evaluate task motivation and motor ability during radial arm maze. The four study groups ($n = 8$ in each group) are as indicated previously: group C, isoflurane anesthesia without surgery; group IL, isoflurane anesthesia with laparotomy; and isoflurane anesthesia with laparotomy plus postoperative analgesia with ketoprofen (IL + ketoprofen or morphine (IL + morphine). *Each vertical bar* represents the mean ± SEM

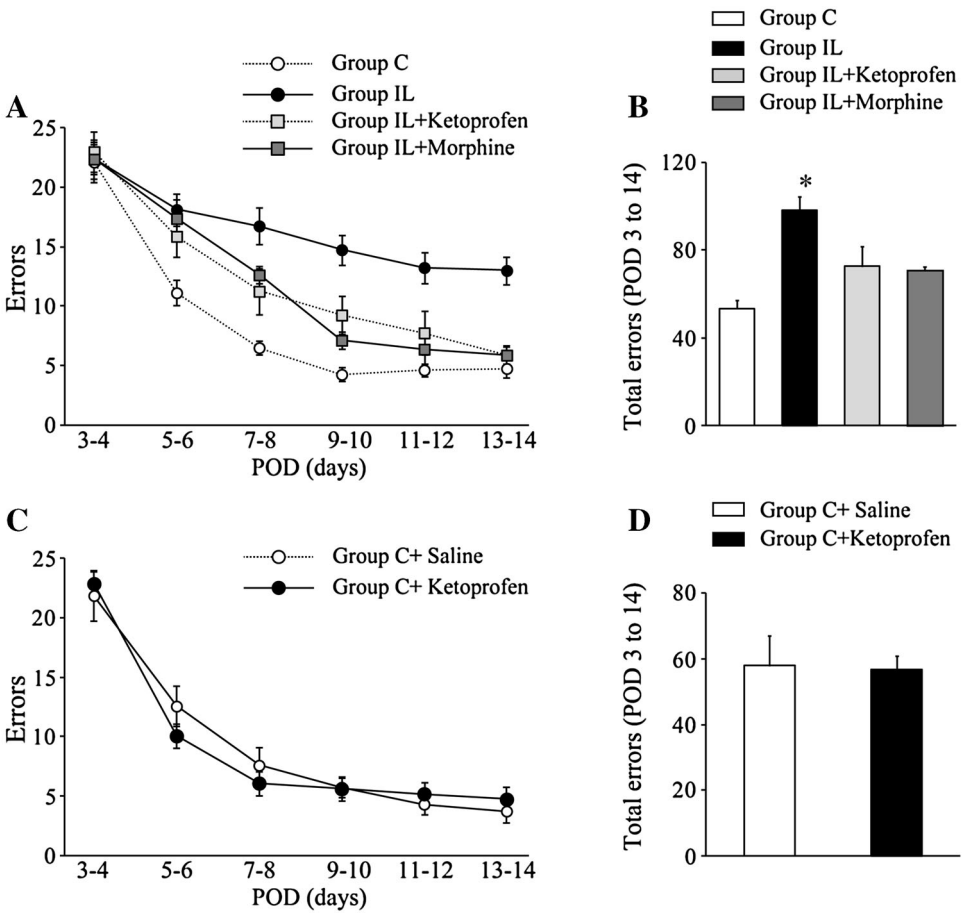

Fig. 2 Spatial memory performance in the 12-radial arm maze in aged rats. **a**, **b** Effects of either ketoprofen or morphine for postoperative acute pain in surgical rats. **c**, **d** Effects of ketoprofen in nonsurgical control rats. The results indicate the group mean ± SEM for memory errors from POD 3 to 14 in each group. The study groups ($n = 8$ in each group) are as indicated previously. *Left panel* (**a**, **c**): Each *point* represents the mean values for 2-day blocks of trials. *Right panel* (**b**, **d**): Total errors over the entire testing period. *$p < 0.05$ vs. group C

change. Locomotor activity was evaluated using the open-field test on postoperative day (POD) 3 by the total accumulated count of horizontal beam crosses in 60 min.

Cognitive function was assessed by a standard radial arm maze for 12 consecutive days (from POD 3 to 14), as previously described [4]. Briefly, the maze consisted of 12 equally spaced radial arms (50 cm long and 11 cm wide, with 20-cm transparent wall) and a common circular center platform 34 cm in diameter. A reward pellet (45 mg) was placed at the end of each arm within a plastic food cup. Six arms were randomly assigned to be baited, and the remaining 6 arms were never baited. The maze was surrounded by multiple visual cues: posters, door and windows, a chair, and some desks. During the trial, rats were placed in the center of the maze individually and allowed to freely explore the maze until all six reward pellets in the food cups had been consumed. Each session was terminated when the rat retrieved all reward pellets or after 15 min had elapsed. The number of errors, defined as the number of entries to nonbaited arms (reference memory) plus re-entries to either baited or nonbaited arms (working memory) were recorded and analyzed in 2-day blocks.

All data were expressed as the mean ± standard error of the mean (SEM). For each dependent variable, we tested the groups with repeated-measures analysis of variance (ANOVA). Whenever ANOVA indicated a statistical significance in identifying a significant main effect, post hoc comparisons between groups followed in a pairwise manner by Wilcoxon–Mann–Whitney test with Bonferroni's correction. $p < 0.05$ was considered statistically significant.

There were no significant differences between the groups for arterial oxygen saturation level, pulse rate, and body temperature at baseline and during and after the inhalation period. Figure 1a indicates the changes in mean RGS scores for acute postoperative pain in each group. No differences in RGS scores were observed in group C, whereas a significant increase in RGS pain score from baseline until 8 h after surgery was observed in group IL. This postoperative increase in RGS pain score was inhibited by both ketoprofen (group IL + ketoprophen) and morphine (group IL + morphine) with similar potency. However, there were no intergroup differences in spontaneous locomotor activity at POD 3 (Fig. 1b), or in average latency per arm choice during the maze trial (Fig. 1c). These results indicate that motor ability and task motivation during the trial were comparable between groups.

All groups showed continual improvement of performance in spatial memory (main effect of POD,

$F_{(3,28)} = 9.49$, $p < 0.01$) tasks across testing periods (Fig. 2a). Repeated-measures ANOVA showed a significant main effect of group ($F_{(15,140)} = 4.66$, $p < 0.05$). Subsequent pairwise comparisons with the Bonferroni correction revealed that the total number of errors in group IL was significantly higher than that in group C (Fig. 2b). This finding indicates that laparotomy resulted in a long-lasting spatial memory deficit. Conversely, effective postoperative pain management with two different analgesics, either ketoprofen or morphine, similarly attenuated the memory deficit after laparotomy. On the other hand, anesthesia-only rats that received ketoprofen did not show any significant effects in spatial memory function compared to vehicle-treated rats (Fig. 2c, d).

Ketoprofen is a representative long-acting NSAID that is frequently used for treatment of moderate to severe acute pain in both humans and laboratory animals. Our results also show that administration with ketoprofen inhibited an increase in the postoperative pain scale based on facial expressions equivalent to that of morphine in an aged-rat laparotomy model. Furthermore, ketoprofen could reverse laparotomy-induced deterioration of performance in radial arm maze task performance without any effect on locomotor activity and motivation. These findings indicate that effective postoperative pain management with ketoprofen may have benefits for preventing the development of POCD in elderly patients.

In the present study, ketoprofen showed no influence on cognitive function in nonsurgical rats that did not experience pain. In addition, our previous study using identical experimental conditions indicated that isoflurane anesthesia alone had no effect on radial maze task performance [4]. Furthermore, similar to ketoprofen, an equianalgesic dose of morphine could prevent surgery-induced memory deficit. These results suggest that ketoprofen prevented the development of POCD via its pain-relieving effects per se, rather than through its direst pharmacological action on cognitive function. This view is partly concordant with previous clinical observations showing that the incidence of POCD was not influenced by the type of postoperative analgesic technique [11].

In the present study, the RGS pain assessment revealed that ketoprofen and morphine similarly produced almost complete analgesia (Fig. 1a), whereas the preventive effects of both analgesic regimens on POCD were partial (Fig. 2a, b). These findings imply that pain-independent mechanisms could also exist and contribute to the development of POCD, so further investigation is needed to clarify the role of such mechanisms. Moreover, we used simple laparotomy without visceral manipulation as a postoperative pain model. The benefit of this model is that it preserves food-seeking and locomotor activity, both of which are essential for subsequent cognitive task performance. However, because this model replicates only minimally invasive surgery, it may not accurately reflect the entire clinical situation. Thus, we cannot rule out the possibility that NSAIDs could not show antiinflammatory effects because of a relatively low inflammatory response in our model. Therefore, the preventive effects of NSAIDs, as well as another class of analgesics, on the development of POCD in more invasive surgical models should be assessed in the future.

Our study has the following limitations. First, because this study was not designed to compare between young and aged rats, it is unclear whether pain-related POCD is specific to aged animals. Second, the dose of ketoprofen used in this study was optimal for the inhibition of RGS after laparotomy. However, as this dose was markedly higher than those currently recommended it may exceed the threshold for gastrointestinal and renal toxicity [12]. Therefore, it may be difficult to directly extrapolate our results to clinical practice.

In conclusion, effective postoperative pain control with ketoprofen could prevent the development of memory deficits following anesthesia and surgery in aged rats. Our findings further suggest that adequate pain relief, independent of analgesic methods, may be important for preventing the development of POCD in elderly patients.

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