



## Original article

## Thalidomide alleviates postoperative pain and spatial memory deficit in aged rats

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## ABSTRACT

Pain is a major risk factor of post-operative cognitive dysfunction (POCD) in aged population. We investigated the effects of thalidomide, an anti-inflammatory and analgesic drug, on POCD in aged rats, and also explored the underlying mechanisms. Laparotomy was performed under anesthesia in aged rats (24–25 months) to establish POCD models. Thalidomide (5–50 mg/kg) was intraperitoneally administered immediately after laparotomy. Within 12 h after the operation, pain symptoms were assessed by rat grimace scale (RGS). Within postoperative day (POD) 3–14, spatial memory was evaluated using performance errors in a radial arm maze. Protein levels of inflammatory cytokines and N-methyl-D-aspartate (NMDA) receptors were measured on POD 14. POCD rats treated with thalidomide showed decreased RGS and performance errors, compared with saline-treated POCD rats. Single administration of thalidomide significantly reduced production of cytokines (tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1 $\beta$ ) in serum but not in the brain, and attenuated upregulation of NMDA receptor (NR) 2A/B subunits in the hippocampus at POD 14. MK-801 abolished thalidomide-induced attenuation of spatial memory deficits. Our results support that thalidomide could disrupt the development of post-operative memory deficit in aged rats through its long-term regulation of NMDA receptors (NRs) in the hippocampus. Therefore, thalidomide might provide a new means to prevent the development of POCD.

## 1. Introduction

Patients subjected to anesthesia and surgery show attention deficits, mild cognitive impairment, and spatial memory dysfunction. These syndromes can be categorized as postoperative cognitive dysfunction (POCD) [1]. POCD has higher prevalence in elderly patient, 14% of which experience cognitive decline and confusion up to 4 months after surgery [2,3]. The risk factors include ageing, preoperative stress, drugs, trauma injury, and postoperative pain [1].

Although pathophysiology of POCD is uncertain, management of postoperative pain is one of options proved effective to prevent POCD [4]. One clinical study revealed that elderly patients with non-cardiac surgery who received postoperative anesthesia is less likely to experience POCD [5]. Similarly, postoperative acute pain is found to exacerbate memory deficits after laparotomy in aged rats [6].

The goal of post-operative pain management is to relieve pain while minimizing side-effects. Although opioids are on the front line of effective analgesics, they also carry many undesirable side-effects, including sedation, respiratory depression, nausea and vomiting, overdose, and addiction [7]. Non-steroidal anti-inflammatory drugs

(NSAIDs) are useful for postoperative pain management, especially for mild and moderate, but not severe pain [8]. Therefore, new and more effective treatment modalities are needed for postoperative pain management.

Thalidomide was first introduced in 1957 as a non-barbiturate sedative drug for the treatment of sleep disorders and morning sickness. Because thalidomide inhibits the synthesis and expression of multiple cytokines including interleukine-1 $\beta$  (IL-1 $\beta$ ), IL-6, granulocyte macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [9], it has been used in inflammatory disorders and cancers [10,11]. Consistent with the notion that inflammation plays key roles in pain sensitization [12], thalidomide effectively relieves complex regional pain syndrome [10] and neuropathic pain, such as chronic sciatic nerve injury, lumbar 5 ventral root transection neuropathy, and chemotherapy-related pain [11,13,14].

Recent studies indicate that inhibition of systemic inflammatory process may interrupt pathogenesis of POCD [15,16]. In the present study, we revealed that thalidomide could disrupt the development of post-operative memory deficit in aged rats through its long-term regulation of NMDA receptors (NRs) in the hippocampus. Therefore,

Abbreviations: POCD, postoperative cognitive dysfunction; RGS, grimace scale; POD, postoperative day; NMDA, N-methyl-D-aspartate; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL, interleukin

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## 2. Methods

### 2.1. Animal

All animal care and experimental protocols were approved by Animal Care and Research Committee in The Central Hospital of Shaoxing City. Aged male Wistar rats (24–25 months old, 550 and 640 g) were used in this study. All rats were housed at  $22 \pm 2^\circ\text{C}$ , 45–75% relative humidity with 12 h light–dark cycle, and water and food were provided *ad libitum*.

### 2.2. Anesthesia, laparotomy and drug treatment

Rats were randomly divided into 5 experimental groups ( $n = 10$  rats/group): A sham group received anesthesia, but without laparotomy; other 4 surgery groups all received anesthesia and laparotomy, and by the end of these treatments, individual surgery groups were respectively subjected to single intraperitoneal injection of saline (vehicle), 5, 20, or 50 mg/kg thalidomide.

Simple laparotomy was performed according to previously reported methods [6]. Briefly, anesthesia was induced with 3% isoflurane, and later maintained with 1.2% isoflurane for 2 h. A 1.0-cm midline longitudinal incision was made through the skin, the abdominal muscle, and the peritoneum. The muscle layers were sutured with 5-0 Vicryl sutures, and the skin was closed with tissue adhesive glue.

Thalidomide and MK-801 were purchased from Sigma Aldrich (USA), and were dissolved in saline. MK-801 (0.1 mg/kg) was given intraperitoneally (*i.p.*) before surgery; saline or thalidomide was given *i.p.* after laparotomy.

### 2.3. Pain measurement

Acute postoperative pain intensity was determined using a rat grimace scale (RGS) as previously reported [17]. Briefly, rats were acclimated in a clear plastic cage with a camera immediately outside of one wall for 10 mins. The activity of the rats was digitally videotaped during acclimation phase. Frame of front view of the head was grabbed randomly. Each frame should include the head with most of action units visible (i.e., nose, cheek, whiskers, eyes, and ears). Five facial features as indices of pain were defined: orbital tightening, nose/cheek fluttering, ear position, and whisker changes. Pain intensity ratings were coded using a three point scale from 0 (not present) to 2 (severe) to facial features presented.

### 2.4. Open field test for measurement of locomotion

Rats were subjected to open field test on postoperative day 3. They were placed in a rectangle chamber, which was equipped with photo-beams. The activity of the rats was quantified as total count of beam breaks in 60 mins.

### 2.5. Radial arm maze for measurement of spatial memory

A standard radial arm maze was used to measure spatial memory, as previously reported [18]. Briefly, the maze contains a central platform with a diameter of 34, which was surrounded with a Plexiglass wall and 12 radial arms (50 cm long and 11 cm wide, with 20 cm transparent wall). A reward pellet (45 mg) was placed randomly at the end of 6 out of 12 arms in a plastic cup. Rats were first acclimated in maze for 2 days. During the trial, rats were placed in the center of the maze and allowed to explore all arms. The following data were record: the latency for per arm choice; the arm (reward or non-reward) the rat entered each time; the total time spent for obtaining all 6 food rewards; number of

correct arm choices (the first visit to reward arms); number of incorrect arm choices (the first visit to non-reward arms).

### 2.6. Western blot

Protein from the hippocampus, the PFC, and the amygdala was extracted for standard western blot assay. Rabbit anti-NR2A/B (1:500, Millipore, Billerica, MA, USA) and mouse anti-GAPDH (1:10000, Abcam, Cambridge, MA, USA) were used as primary antibodies. Antibody–protein complexes were visualized by chemiluminescent reagents. Band density was quantified by imaging quantification. Ratios of the band density for the protein of interest to that for GAPDH were calculated.

### 2.7. ELISA assay of TNF- $\alpha$ and IL-1 $\beta$

TNF- $\alpha$  and IL-1 $\beta$  in serum and brain were measured using ELISA ready-SET-Go!<sup>®</sup> assay kits (eBioscience, San Diego, USA), according to the instruction provided by the manufacturer. Protein level was expressed as pg/ $\mu\text{g}$  of total proteins determined over an albumin standard curve.

### 2.8. Statistical analysis

Data are presented as mean  $\pm$  SD. Statistical significance was determined by one- or two-way ANOVA with Dunnett's or Tukey's post-hoc tests using the GraphPad Prism<sup>®</sup> 5 software, depending on whether the data passes the normality test. Difference was considered statistically significant when *P* values were less than 0.05.

## 3. Results

### 3.1. Effect of thalidomide on postoperative acute pain in aged rats

Rat grimace score (RGS) was used to evaluate the intensity of pain. Thalidomide (5–50 mg/kg) was administrated by *i.p.* injection after laparotomy. Compared with sham group, all surgical groups developed significant acute pain starting 2 h postoperatively, evidenced by higher RGS ( $p < 0.05$ , One-way ANOVA), and recovered to baseline level 12 h after surgery ( $p > 0.05$ , One-way ANOVA) (Fig. 1a). The concentration-response relationship showed (Fig. 1a) that single injection of thalidomide attenuated pain intensity in a dose-dependent manner, with statistical significance at doses of 20 and 50 mg/kg ( $p < 0.01$ ). Additionally, thalidomide at 20 and 50 mg/kg induced a faster recovery of postoperative pain, compared with saline treatment ( $p < 0.05$ ) (Fig. 1a). These data suggest that single administration of thalidomide has anti-analgesic effect on acute pain following laparotomy.

Open-field test on post-operative day 3 (POD 3) showed that there was no difference in locomotor activity among experimental groups ( $p > 0.05$ ) (Fig. 1b). Time lapsed before arm choice in the maze was analyzed for evaluation of task motivation. As shown in Fig. 1c, latency for arm choice did not differ between groups ( $p > 0.05$ ). These data indicate that neither surgery nor thalidomide treatment changed locomotion and task motivation in aged rats.

### 3.2. Effect of thalidomide on spatial memory performance

Spatial memory performance was analyzed using a radial arm maze from POD 3–14. Compared to non-surgical sham group, the working memory errors in all surgical groups were significantly higher than those in sham group from POD 5–14 ( $p < 0.05$ ) (Fig. 2a, b), suggesting that laparotomy resulted in spatial memory deficit. Single injection of thalidomide by the end of surgery significantly reduced memory errors in a dose-dependent manner at POD 8, 10, 12, 14, with significant difference at 20 and 50 mg/kg ( $p < 0.05$ ) (Fig. 2a, b). The results suggest that thalidomide not only effectively relieved postoperative

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