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Cerebral mast cells contribute to postoperative cognitive dysfunction by promoting blood brain barrier disruption

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HIGHLIGHTS

- Activated mast cells are involved in neuroinflammation and cognitive dysfunction following surgery by promoting BBB disruption.
- Mast cell stabilizer disodium cromoglycate alleviated surgery-induced cognitive decline and neuroinflammation.
- Disodium cromoglycate inhibited the increase of BBB permeability induced by surgery.

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ABSTRACT

Trauma induced neuroinflammation plays a key role in the development of postoperative cognitive dysfunction (POCD). The blood–brain barrier (BBB), a highly specialized endothelial layer, is exquisitely sensitive to inflammatory insults, which can result in numerous neurocognitive syndromes. While brain mast cells are the "first responder" in the injury, the functional interactions between mast cells and the BBB remain poorly understood. Our results demonstrate that tibial fracture surgery can induce cognitive impairment relating to an inflammatory response and destabilization of the BBB. Disodium cromoglycate (cromolyn) – which acts as a mast cell stabilizer – inhibited this effect. Specifically, cromolyn resulted in ameliorated cognitive ability, decrease of inflammatory cytokines and increase of BBB stability. Taken together, these results suggest that activated mast cells contributed to central nervous system inflammation and cognitive dysfunction by promoting BBB disruption, and interactions between mast cells and the BBB could constitute a new and unique therapeutic target for POCD.

1. Introduction

Postoperative cognitive dysfunction (POCD) is a common complication frequently seen among elderly patients following surgery and hospitalization [1]. Although the pathogenesis of POCD remains unclear, neuroinflammation initiated by extra-central nervous system (CNS) surgical trauma has been proposed as a key component of surgery-induced cognitive dysfunction [2]. Surgery exerts an effect on the CNS by releasing inflammatory mediators [typically tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, and muted anti-inflammatory responses from IL-4, IL-10, and tumor growth factor (TGF)-b], which communicate with the CNS by vagal afferents and cross the blood–brain barrier (BBB). Together these cytokines result in a neuroinflammatory response, which may damage synapses and neurons, and ultimately lead to POCD [3].

The BBB is a multicellular vascular structure that separates the CNS from the peripheral blood circulation. By protecting the brain from toxins and pathogens, the BBB maintains an environment that allows neurons to function properly. Physiologically, endothelial cells (ECs) of the BBB form continuous intercellular network of tight junctions (TJs). TJs possess a matrix metalloproteinase (MMP) cleavage site that can lead to a breach in the BBB via detachment of endothelial cells from the extracellular matrix (ECM). It has been suggested that reduced BBB function may contribute to the cognitive dysfunction associated with aging [4]. Based on recent findings, it has been suggested that surgery adversely affects the BBB permeability and function [5]. On the other hand, activation of MMPs after ischemic stroke could also increase BBB permeability by decreasing the expression of claudin-5 and occludin, the key components of TJs [6]. Thus, we hypothesized that alteration of MMPs and TJs were involved in POCD following surgery.

Abbreviations: POCD, postoperative cognitive dysfunction; BBB, blood–brain barrier; CNS, central nervous system; ECs, endothelial cells; TJs, endothelial cells; MMP, matrix metalloproteinase; ECM, extra cellular matrix; EB, Evans blue; TB, toluidine blue; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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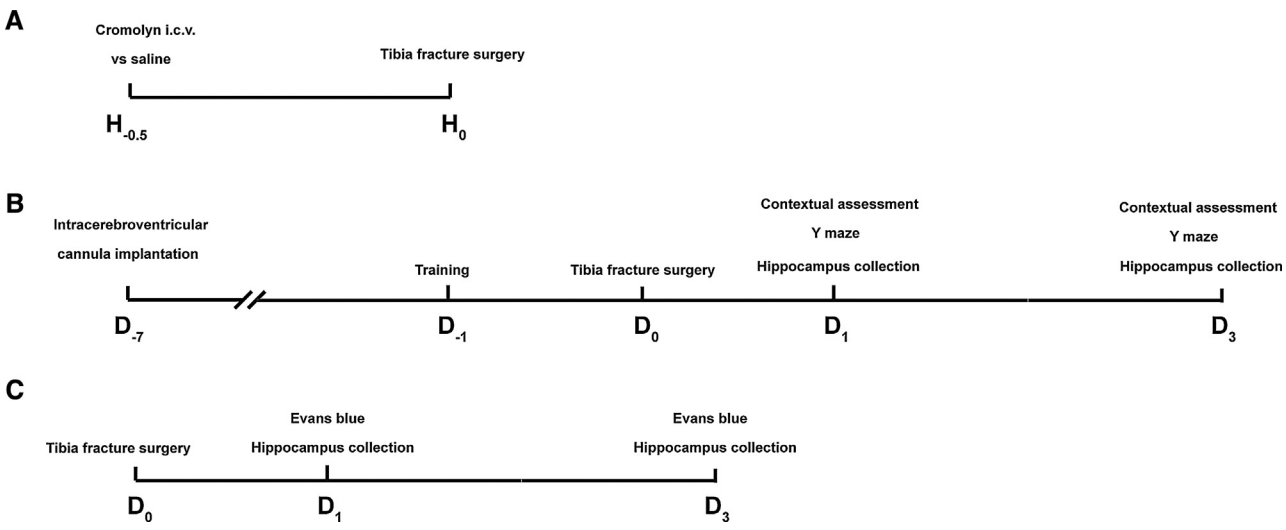

Fig. 1. Study design. (A) Drug treatment: of the four groups, Cro group and Cro + op group received cromolyn *i.c.v.* 30 min before surgery, while the others received an equivalent volume of normal saline. (B) Experiment 1: All rats received *i.c.v.* cannula implantation 7 days before used. One day after contextual fear conditioning training, all animals were divided into four groups and received cromolyn or saline treatment following tibial fracture surgery, where applicable. All animals received contextual assessment and Y maze test 1 and 3 days after surgery. Brains were collected after behavior tests. (C) Experiment 2: After behavioral tests. Evans blue (EB) was injected intravenously and allowed to circulate for 60 min, then the hippocampus were collected for calculating of EB extravasation.

Although numerous POCD-focused studies have investigated the interactions with neurons and glial cells, few studies reported an investigation of the impact of mast cells. Mast cells represent a potentially important and underappreciated peripheral immune signaling link to the brain during an inflammatory insult [7]. Mast cells, previously implicated in immunoglobulin type E (IgE)-associated allergic and inflammatory disorders, are distributed in a variety of anatomical sites, including the CNS, where they are located perivascularly in proximity to neurons and microglia [8,9]. Mast cells produce a number of inflammatory mediators, including biogenic amines (histamine and serotonin), cytokines (IL-1–IL-6, TNF-α and so on), enzymes, lipid metabolites, ATP, neuropeptides, growth factors, nitric oxide, and heparin [10]. In the absence of stress, disease, or trauma, the number of mast cells is considerably smaller than that of neurons, microglia, and other cells of the CNS. However, despite their small numbers, activated mast cells can have a dramatic effect on the BBB, neurons, microglia, and astrocytes. The multiphasic response pattern of mast cells, wherein they release preformed granular material within minutes and newly synthesized mediators for the next several hours, enables their actions as catalysts that amplify and prolong numerous vasoactive, neuroactive, and immunoactive cellular and molecular responses [11]. Moreover, mast cells located within the CNS capture and respond to immune signals from the blood even faster than microglia [12,13]. Substantial evidence suggests that mast cells can promote BBB breakdown [14,15]. We believe that the effects of mast cells may drive BBB breakdown and thereby impact cognitive dysfunction secondary to peripheral surgery.

In this study, we used a tibial fracture surgical model in adult rats to define the role of mast cells in mediating surgery-induced cognitive dysfunction and further clarify the immune-to-brain signaling, that may associate with cognitive decline.

2. Materials and methods

2.1. Animals

A total of 128 adult male Sprague-Dawley rats, weighting 200–250 g, were purchased from Jinling Hospital of Nanjing University. All rats were housed in groups of five per cage and maintained under standard environmental conditions (12 h light/dark cycle, 22 ± 1 °C and 55 ± 5% humidity) during the experimental period, with water and food available ad libitum. This study was approved by the Nanjing Medical University Animal Care and Use Committee. All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health of the United States. Study design was briefly demonstrated in Fig. 1.

2.2. Intracerebroventricular cannula implantation

For the intracerebroventricular (*i.c.v.*) administration of drugs, rats were placed in the stereotaxic apparatus (Stoelting Instruments, USA) after they had been anesthetized. A 21-gauge stainless steel guide cannula was implanted into the right lateral ventricle using the following coordinates: 0.8 mm posterior, 1.5 mm right lateral, and 3.7 mm ventral to the bregma. The guide cannula was secured by dental cement, anchored by stainless steel screws fixed to the skull. The rats were allowed to recover for 7 days and housed individually before experimental use. Animals were handled daily to check the guide cannula and to familiarize them with the investigators. At the time of drug administration, a 29-gauge injection cannula connected to a microsyringe pump by a PE-20 catheter was filled with drug solution and inserted into the guide cannula extending 1 mm beyond the guide cannula tip. The needle was maintained in this position for an additional 5 min after injection and then retrieved slowly from the brain.

2.3. Drug treatment and surgical procedure

All rats were randomly allocated to four groups: (i) *i.c.v.* injection of normal saline (Ctrl group); (ii) *i.c.v.* injection of cromolyn (Cro group); (iii) tibial fracture surgery following *i.c.v.* injection of normal saline (Op group); (iv) tibial fracture surgery following *i.c.v.* injection of cromolyn (Cro + op group). Rats in the Cro group and Cro + op group received 200 μg/2 μl cromolyn *i.c.v.* 30 min before surgery, while the others received an equivalent volume of normal saline.

We performed open tibial fracture surgery as previously described [16,17]. Under general anesthesia with isoflurane (2.1%