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Propofol administration improves neurological function associated with inhibition of pro-inflammatory cytokines in adult rats after traumatic brain injury

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ABSTRACT

Background: Neurological deficits following traumatic brain injury (TBI) result in dramatic impacts on the survivors, but the effect of propofol and associated mechanism are waiting to be determined.
Methods: Adult male Sprague–Dawley rats were randomly assigned into Sham, TBI, TBI + Intralipid and TBI + Propofol group. Modified Feeney method was adopted to generate TBI model from free hammer fall injury, and animals in TBI + Propofol group were immediately treated with propofol administration for 2 hours after TBI, rats after TBI without propofol treatment was used as injury control, intralipid as vehicle in propofol was injected in TBI + intralipid group. Then, neurological severity scores (NSS) were evaluated at 1, 3, 7 and 14 days. Moreover, the expressions of IL-1β, IL-6 and TNF-α mRNA and protein were examined using quantitative real time-polymerase chain reaction and Western blot, immunohistochemical staining was used to localize cytokines.
Results: The NSS increased greatly in the rats induced by TBI, while propofol could effectively decreased NSS, confirming the neuroprotective effect of propofol. Moreover, the mRNA expressions of IL-1β, IL-6 and TNF-α, at 1, 3, 7 days after operation (dpo), were significantly augmented in the injured cortex, compared with sham one. But there was no difference between TBI and TBI + Intralipid group, but markedly decreased after propofol treatment. Additionally, the protein level of IL-1β, IL-6 and TNF-α in four groups determined by Western blot and immunohistochemistry showed the similar change with mRNA expression.
Conclusion: Propofol treatment could elicit a robust neuroprotective response, resulting in significant neurological function improvement for TBI rats, which was independent with intralipid. The underlying molecular mechanism may be partially associated with an inhibition of pro-inflammatory cytokines.

1. Introduction

Traumatic brain injury (TBI), defined as direct mechanical damage to the brain (Prins et al., 2013), is a leading cause of mortality and morbidity in the population all over the world (Andelic, 2013; Mauritz et al., 2014; Cuthbert et al., 2014). Neurological deficits and cognitive decline following TBI commonly occurred and resulted in dramatic impacts on these survivors, which has become a significant public health problem (Scholten et al., 2014; Gubata et al., 2014). Although extensive efforts have been done to develop neuroprotective therapies for TBI, there have been no successful treatment which could effectively improve cognitive and behavioral deficits to date (Kabadi and Faden, 2014).

Brain damage following TBI is induced by primary mechanical insult and secondary mechanisms that involves activation of proinflammatory cytokines, neuronal loss, cerebral edema, finally lead to neurological dysfunction (Prins et al., 2013). Acute inflammatory response within the injured brain has shown to be an important role in the development of secondary brain injury (Goodman et al., 2008; Ziebell and Morganti-Kossmann, 2010). The proinflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) significantly elevate after TBI, which has been considered as important mediators for the initiation and the support of post-traumatic inflammation, thus caused additional cell death and neurological dysfunction (Lenzlinger et al., 2001). Because of the key mechanism of neuroinflammation in the development of delayed injury post TBI, a number of treatments via anti-inflammatory mechanism have been shown to prevent progressive tissue damage and loss of function after injury in animal models (Cordaro et al., 2014; Zhu et al., 2014; Kumar and Loane, 2012; Zhang et al., 2013).

Propofol (2,6-diisopropylphenol), a short-acting intravenous anesthetic agent, is widely used for the treatment of head injury, including

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anesthesia for surgical intervention and sedation in the intensive care unit (Fulton and Sorkin, 1995). Propofol has been shown to exert neuroprotective effect in many established experimental models of acute cerebral ischemia/reperfusion injury both in vivo and vitro (Vasileiou et al., 2009; Kotani et al., 2008). However, experimental studies on TBI are limited and less encouraging. In vitro, propofol has been found to protect rat microglial cells against extracellular pressures, stimulating phagocytosis, proliferation, TNF-α, interleukin-1β (IL-1β), and total nitrate secretion (Yu et al., 2011). In vivo, following propofol treatment in the rats after TBI within a short time, Ding found that propofol significantly reduced brain edema in a controlled cortical injury rat model and suppressed the expression of AQP-4 associated with attenuated expression of IL-1β and TNF-α (Ding et al., 2013). Luo found that propofol improved cognitive recovery after TBI in novel object recognition test associated with limited microglial activation through inhibition of nicotinamide adenine dinucleotide phosphate oxidase (Luo et al., 2013). However, other animal and human studies didn't support above findings. Thal found that propofol infusion during controlled cortical impact induction or 2 h after TBI resulted in aggravation of neurological dysfunction, increased 28-day mortality rate, and impaired posttraumatic neurogenesis (Thal et al., 2014). Furthermore, clinical studies comparing propofol with other anesthetics or sedatives could not provide strong evidence to indicate that propofol may be superior to other anesthetics in improving the neurological outcome following acute cerebral injury. Therefore, the role of propofol in TBI model needed to be explored, and the mechanism involving in brain protection is waiting to be further investigated.

In this study, we investigated the neuroprotective effects of propofol in rats following TBI, determined by neurological severity scores and expression of pro-inflammatory cytokines in the injured cortex.

2. Materials and methods

2.1. Animals and groupings

212 adult male Sprague–Dawley rats weighing 200 ± 20 g, supplied by Kunming experimental animal center, were randomly assigned into four groups (53 in each group): Sham control group (N), TBI group (T), TBI + Intralipid group (TI), TBI + Propofol group (TP). In TP group, rats were treated with propofol 100 mg/kg, which was intraperitoneally injected immediately after TBI model was established. In TI group, rats were treated with 10 ml/kg intralipid at the same time with TP group. All the rats raised on a temperature- and humidity-controlled condition under a 12 h light/dark cycle, with free access to food and water. Animal groups and experiment technology were shown as following (Table 1).

2.2. Anesthesia procedure and TBI model

All the rats were fasted for 12 h before surgery. The rats were sedated with spontaneous breath under intraperitoneal injection of 3.6% chloral hydrate (10 ml/kg) and fixed on a stereotactic platform in the prone position. Modified Feeney method was adopted to establish the traumatic brain injury model. A 5-mm craniotomy was created in the skull 1.5 mm behind the coronal suture and 2.5 mm beside the midline. After removing the skull, the right parietal cortex was exposed with the dura intact. Thereafter, the animals in T, TI and TP group were impacted following 50 g weight falling freely in the height of 25 cm which resulted in injury of the right parietal cortex. The impact was approximately equal to a moderate TBI in humans. After injury, the craniotomy was closed.

Table 1
Animal used.

Model	NSS (1, 3, 7, 14 dpo)	Q-PCR (1, 3, 7 dpo)/WB (3 dpo)	IHC (3 dpo)
Sham	10	10 × 4	3
TBI	10	10 × 4	3
TBI + Intralipid	10	10 × 4	3
TBI + Propofol	10	10 × 4	3

2.3. Assessment of neurological function

Rats were assessed before TBI and on 1st, 3th, 7th, and 14th day after TBI by three investigators who were blinded to the experimental groups by using modified neurological severity scoring (NSS) (Chen et al., 2001). NSS is a complex behavioral test including motor, sensory, reflex and balance tests, which has a scoring range of 0–18, with higher scores reflecting greater extent of injury (Table 2). A score of 0 indicates sham performance, a score of 18 indicating maximal impairment, 13–18 points indicate severe injury, 7–12 indicate mean-moderate injury, and 1–6 indicate mild injury.

2.4. Tissue preparation

Rats were anesthetized on corresponding time point after TBI with intraperitoneal injection of 3.6% chloral hydrate (10 ml/kg), the brain tissue within 5 mm radius around the injury center were rapidly removed and stored frozen at −80 °C for the real time-PCR (1, 3, 7 dpo) and Western blot analysis (3 dpo).

For immunohistochemistry, transcardial perfusion with 250 ml 0.9% saline was adopted after anesthesia, followed by 300 ml of 4% paraformaldehyde, then the brain was taken out on 3 dpo and dehydrated in the 15% sucrose solution for 12 h, followed by dehydration in 30% sucrose solution.

2.5. Expression of IL-1β, IL-6 and TNF-α in injured cortex tissue by Using quantitative RT-PCR

Quantitative RT-PCR was performed as previously (Yisarakun et al., 2015). Primers of IL-1β, IL-6 and TNF-α were designed with the primer 5.0 soft-ware and then empirically tested (Table 3). Total RNA was

Table 2
Neurological severity scores (NSS).

	Point
Motor tests	
Raising rat by the tail (normal = 0; maximum = 3)	
Flexion of forelimb	1
Flexion of hindlimb	1
Head moved >10° to vertical axis within 30 s	1
Placing rat on the floor (normal = 0; maximum = 3)	
Normal walk	0
Inability to walk straight	1
Circling toward the paretic side	2
Fall down to the paretic side	3
Sensory tests (normal = 0; maximum = 2)	
Placing test (visual and tactile test)	1
Proprioceptive test (deep sensation, pushing the paw against the table edge to stimulate limb muscles)	2
Beam balance tests (normal = 0; maximum = 6)	
Balances with steady posture	0
Grasps side of beam	1
Hugs the beam and one limb falls down from the beam	2
Hugs the beam and two limbs fall down from the beam, or spins on beam (>60 s)	3
Attempts to balance on the beam but falls off (>40 s)	4
Attempts to balance on the beam but falls off (>20 s)	5
Falls off: No attempt to balance or hang on to the beam (<20 s)	6
Reflexes absent and abnormal movements (normal = 0; maximum = 4)	
Pinna reflex (head shake when touching the auditory meatus)	1
Corneal reflex (eye blink when lightly touching the cornea with cotton)	1
Startle reflex (motor response to a brief noise from snapping a clipboard paper)	1
Seizures, myoclonus, myodystony	1
Maximum points	18