Increased Expression of Toll-Like Receptor 4 on Neurons After Surgery in Aged Rats

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Postoperative cognitive dysfunction (POCD) is a common complication after surgery in the elderly and is induced by multiple factors including dysregulation of the immune system [1]. Toll-like receptor 4 (TLR4) is a key pattern recognition receptor (PRR) that mediates innate and adaptive immune processes both related to infection, *via* signaling with pathogen-associated molecular patterns (PAMPs), and endogenous danger molecules, or damage-associated molecular patterns (DAMPs) [2]. Within the brain, TLR4 is mainly expressed on microglia and neurons, and changes in its expression have been correlated with several central nervous system (CNS) disease states, such as Alzheimer's disease (AD) and cerebral ischemia [3]. Upon activation, TLR4 signaling pathways lead to neuroinflammation [4], which can further develop into neurodegeneration and permanent cognitive deficits. Yet, there is no clear understanding on whether TLR4 contributes to the neuroinflammatory process of POCD. In this study, we sought to investigate the expression of TLR4 on neurons in the hippocampus after surgery in aged rats. Here, we reveal that high expression of TLR4 on neuron contributes to neuroinflammation after peripheral surgery and general anesthesia.

Female Sprague-Dawley rats (22–23 month old, total $n = 105$) were used in accordance with the guidelines of experimental animal use established by the ethics committee of the Central South University. Animals underwent splenectomy under isoflurane anesthesia for 2 h (group S) or served as anesthesia (group A) and untreated controls (group C), respectively. Brains were harvested at 1, 3 and 7 days. Neurons (NeuN) and TLR-4 expression in the hippocampus was measured using double immunofluorescence combined with measurements of key pro-inflammatory cytokines including TNF- α and IL-1 β in hippocampal homogenate using

Western blot. Data are expressed as mean $\pm$ SD and analyzed by two-way ANOVA (SPSS 17.0, Shanghai, China).

The expression of TLR4 on neurons was significantly up-regulated after surgery, especially on postoperative day 1 compared with control rats (Figure 1C and S1). TLR4 expression was decreased thereafter, with levels returning almost to baseline by postoperative day 7 (Figure 1S7). Remarkably, the expression of TLR4 was also increased after exposure to anesthesia only (Figure 1A), although levels were significantly lower than that after surgery. To corroborate the immunofluorescence findings, we measured TLR4 protein levels with Western blot (Figure 2). We also assessed levels of pro-inflammatory cytokines, IL-1 β and TNF- α , in the hippocampus to further illustrate neuroinflammation after surgery and anesthesia. After splenectomy (Group S), we found a peak in hippocampal protein levels of IL-1 β and TNF- α , returning to baseline by day 7. The increase in IL-1 β and TNF- α after anesthesia exposure (Group A) was not as remarkable as after surgery.

Peripheral surgery triggers both humoral and neuronal changes that cause CNS dysfunction and neuroinflammation [5]. Systemic pro-inflammatory cytokines can enter the brain, disrupt the homeostasis of CNS microenvironment and initiate a local immune response by affecting the blood–brain barrier (BBB) function and permeability [6]. TLR4 is a well-known pro-inflammatory receptor involved in the regulation of the immune response [7]. In the CNS, it has been reported that TLR4 on microglia contributes to abundant production of various pro-inflammatory cytokines *via* MyD88- and TRIF-dependent signaling pathway. Furthermore, TLR4 is also associated with neurotoxicity

Figure 1 Expression of toll-like receptor 4 (TLR4) on neurons in the hippocampus. Representative photomicrographs were taken from CA3 area (scale bar = 50 μ m). Expression of TLR4, neurons (NeuN), and co-localization in all the rats. TLR4 expression after anesthesia, isoflurane exposure, at day 1, 3, and 7 respectively (A1–A3–A7). One day after anesthesia there is a moderate increase in TLR4 expression in neurons (A1), then returning to control levels (A3 and A7). Expression of TLR4 in surgical rats after splenectomy at day 1, 3, and 7 respectively (S1–S3–S7). Surgery causes a significant increase in TLR4 expression up to day 3 (S1 and S3), with some minor changes present also at day 7 (S7).

Figure 2 Western blot results for toll-like receptor 4 (TLR4), IL-1 β and TNF- α protein expression in hippocampal homogenate tissue. Anesthesia exposure caused a significant changing in TLR4, IL-1 β and TNF- α protein levels at day 1, with TNF- α levels remaining elevated up to day 3, then returning to baseline. Surgery caused a further increase in levels of TLR4, IL-1 β and TNF- α both at 1 and 3 days postoperatively. Results are represented as mean $\pm$ SD, $n = 15$ /group. * $P < 0.05$ versus Group C. C: control; A1, A3 and A7: anesthesia on day 1, 3, and 7; S1, S3 and S7: surgery on postoperative day 1, 3, and 7.

and neuroapoptosis, for example, after ischemic brain injury [8]. Few studies have focused on the role of TLR4 in POCD, and the molecular mechanism of TLR4 on neuron in neuroinflammation remains unclear.

In this study, we found that the expression of TLR4 on hippocampal neurons was remarkably increased after surgery. Higher expression of TLR4 on microglia has been previously reported in models of AD, stroke and other CNS disorders. Neuron and microglia closely interact with each other to overall regulate CNS homeostasis and brain function. When the CNS is injured, for example, after surgical trauma, microglia becomes activated ultimately contributing to neuroinflammation and cognitive decline [9]. It is possible that the high expression of TLR4 induced by surgery may also cause neuroapoptosis, and this may trigger microglia activation. Notably, microglia in the aged brain are “primed” and possibly more susceptible to changes in CNS homeostasis [10]. Once activated, microglia produce and release multiple pro-inflammatory cytokines, leading to extensive inflammation, which can finally lead to cognition decline. Gradually after surgery, the expression of TLR4 returned to baseline and also the neuroinflammation disappeared, suggesting that TLR4 activation may regulate microglia activity and brain homeostasis. Our findings indicated that TLR4 on neuron plays a crucial role in modulating surgery-induced neuroinflammation by controlling microglia activation. Of interest, isoflurane anesthesia may also partly contribute to higher TLR4 expression, thus contributing to POCD.

In conclusion, the present study revealed a close relationship between TLR4 and neuroinflammation after surgery, indicating that TLR4 might be a key factor in the development of POCD. To deeply understand the role of TLR4 in POCD, further studies should focus on the relevant molecular mechanism and selective therapies to modulate its expression in the brain.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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