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Selective NLRP3 inflammasome inhibitor reduces neuroinflammation and improves long-term neurological outcomes in a murine model of traumatic brain injury

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ABSTRACT

The nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain containing 3 (NLRP3) inflammasome-mediated inflammatory response has emerged as a prominent contributor to the pathophysiological processes of traumatic brain injury (TBI). Recently, a potent, selective, small-molecule NLRP3 inflammasome inhibitor, MCC950, was described. Here, we investigated the effect of MCC950 on inflammatory brain injury and long-term neurological outcomes in a mouse model of TBI. Male C57/BL6 mice were subjected to TBI using the controlled cortical impact injury (CCI) system. Western blotting, flow cytometry, and immunofluorescence assays were utilized to analyze post-traumatic NLRP3 inflammasome expression and determine its cellular source. We found that NLRP3 inflammasome expression was significantly increased in the peri-contusional cortex and that microglia were the primary source of this expression. The effects of MCC950 on mice with TBI were then determined using post-assessments including analyses of neurological deficits, brain water content, traumatic lesion volume, neuroinflammation, blood-brain barrier (BBB) integrity, and cell death. MCC950 treatment resulted in a better neurological outcome after TBI by alleviating brain edema, reducing lesion volume, and improving long-term motor and cognitive functions. The therapeutic window for MCC950 against TBI was as long as 6 h. Furthermore, the neuroprotective effect of MCC950 was associated with reduced microglial activation, leukocyte recruitment, and pro-inflammatory cytokine production. In addition, MCC950 preserved BBB integrity, alleviated TBI-induced loss of tight junction proteins, and attenuated cell death. Notably, the efficacy of MCC950 was abolished in microglia-depleted mice. These results indicate that microglia-derived NLRP3 inflammasome may be primarily involved in the inflammatory response to TBI, and specific NLRP3 inflammasome inhibition using MCC950 may be a promising therapeutic approach for patients with TBI.

1. Introduction

Traumatic brain injury (TBI) is a worldwide health problem with high mortality and morbidity, and the effective clinical translation of pharmacotherapies for patients with TBI remains insufficient (Ge et al., 2014; Harrison et al., 2015). The pathophysiology of TBI involves a primary mechanical insult and multi-factorial secondary injury cascades (e.g., oxidative stress, apoptosis, and neuroinflammation) (Wang et al., 2016). Accumulating evidence suggests that innate immunity and neuroinflammation are involved in the pathogenesis of TBI (Simon et al., 2017). Upon brain injury, cellular damage results in the rapid release of damage-associated molecular patterns [DAMPs, e.g., ATP, DNA, reactive oxygen species (ROS)] (Corps et al., 2015). The recognition of DAMPs by pattern recognition receptors (PRRs) expressed on cells of the innate immune system then induces the local production of cytokines and chemokines that subsequently facilitate the activation,

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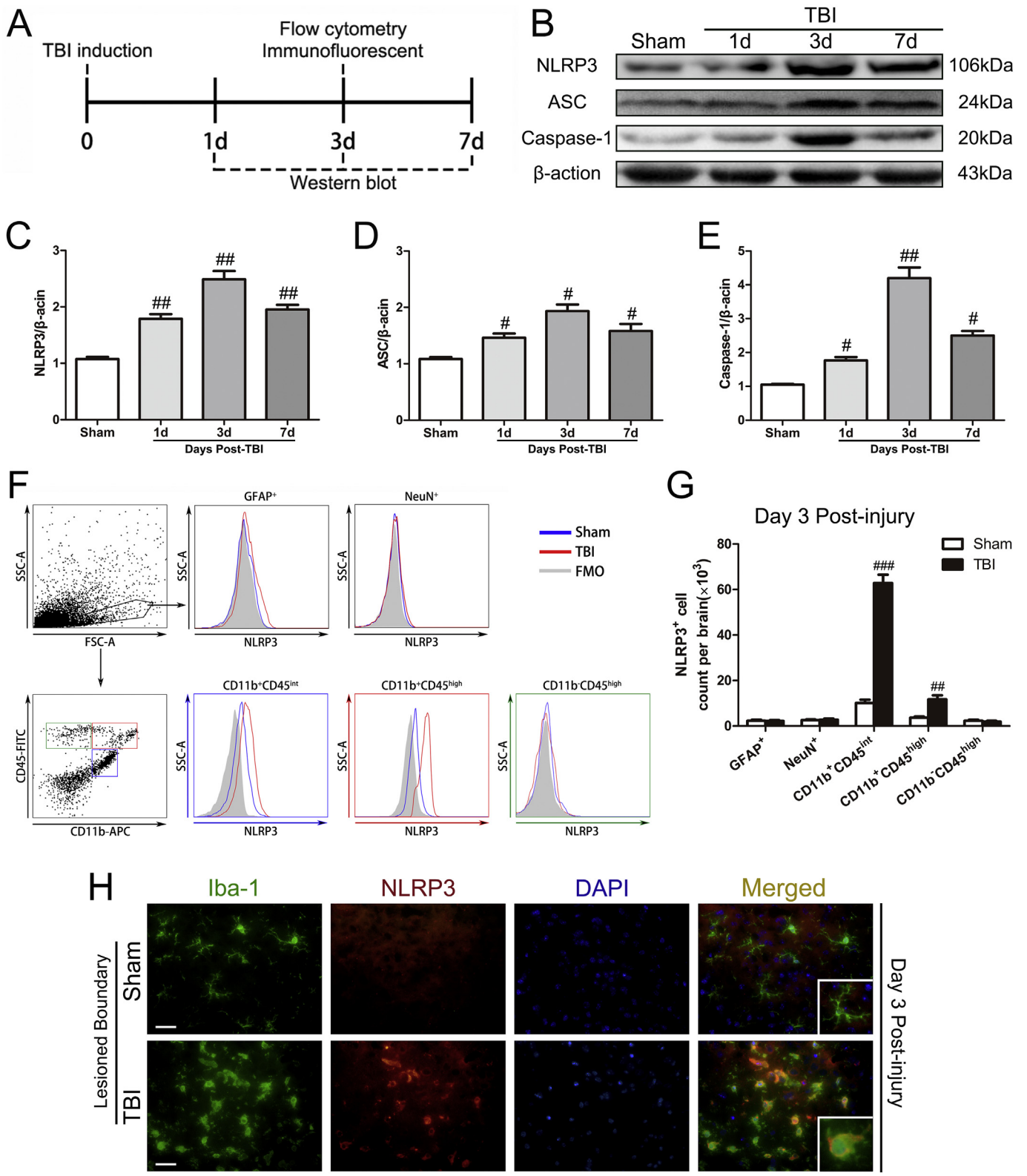

Fig. 1. The time course and cell source of NLRP3 inflammasome expression in the peri-contusional cortex after TBI.
(A) Schematic diagram of the experimental design. (B–E) Representative western blotting bands and densitometric quantification of NLRP3, ASC, and caspase-1 p20 subunit in the peri-contusional cortex from mice subjected to sham surgery or TBI. The expression levels of NLRP3 (C), ASC (D) and caspase-1 p20 (E) were significantly increased at 1, 3, and 7 d post-injury in the TBI group compared with the sham group. Notably, expression levels of these proteins peaked at 3 d post-TBI. (F) The gating strategy of GFAP+, NeuN+, CD11b + CD45int, CD11b + CD45hi, and CD11b-CD45hi cell subsets that express NLRP3. (G) Flow cytometric analysis showed that CD11b + CD45int microglia were the predominant cell subset expressing NLRP3 inflammasome after TBI. (H) Representative photographs of double immunofluorescence staining for Iba-1 (green) and NLRP3 (red) in the peri-contusional cortex at 3 d post-injury. Scale bar = 200 μm. Data are presented as the mean ± SEM. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. sham group. $n = 6$/group.