A novel therapeutic target for intracerebral hemorrhage: interfering with the TLR2/TLR4 heterodimerization

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The investigation of the upstream events that initiate inflammatory responses has a very important role in the illumination of the mechanisms of intracerebral hemorrhage (ICH)-induced secondary inflammatory brain injury. The formation of novel TLR2/TLR4 heterodimer after ICH detected by the western blot, immunoprecipitation and immunofluorescence technologies has strongly contributed to such advance. Using the in vivo and in vitro ICH model, we revealed the assemble of TLR2/TLR4 heterodimer mediated inflammatory injury only can be triggered by the hemoglobin (Hb) via the myeloid differentiation primary response gene 88 (MyD88), rather than toll/IR-1 (TIR)-domain-containing adaptor protein inducing interferon-beta (TRIF). And the mutation site at the MyD88 Arg196 abolished the TLR2/TLR4 heterodimerization. Together, our findings not only further explain the upstream events that initiate inflammatory responses following ICH, but also provide a novel target-interfering with the assembly of TLR2/TLR4 heterodimer-for developing an effective treatment of ICH.

Keywords: Intracerebral Hemorrhage; Inflammation; Toll-like receptors; Heterodimer


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Intracerebral hemorrhage (ICH) is a stroke subtype that characterized by a high morbidity and mortality [1, 2], most of the patients exhibited more severely disabled compared to patients with ischemic stroke [3]. Increasing evidences suggesting that apart from the directly mass effect of hematoma, secondary inflammatory injury contributes to the ICH-induced brain damage that cause severe neurological deficits [4, 5]. Following ICH, the secondary inflammatory injury could be triggered by the components of blood, such as hemoglobin (Hb), heme and fibrinogen, which subsequently activates the innate immune response [5-8] (Figure).

Recent years, TLR4 has been most widely researched in the experimental ICH models [9, 10] and that its signaling pathway plays a critical role in the poor outcome after ICH [10, 11]. Previously research has shown that TLR4 was upregulated from 6 hours through to 3 days in a rat ICH model [9]. In addition, Sansing et al. [10] demonstrated that TLR4^{-/-} mice had significantly decreased perihematomal inflammation and improved functional outcome at day 3 after ICH and they also confirmed that TLR4 expressed on the leukocytes or platelets contributes to perihematomal leukocyte infiltration and causes the neurological deficit, suggesting that TLR4 signaling has a pivotal role in the
perihematoma inflammation and injury. Meanwhile, our previously study also showed that heme activates TLR4 signaling pathway via the MyD88/TRIF to induce the inflammatory injury after ICH [6] and the inflammatory injury effect was blockaded by TLR4 antagonist TAK-242. Hemoglobin(Hb), one of the erythrocyte’s degradation products following ICH, not only triggers the TLR2 to cause inflammatory injury, but also triggers the formation of TLR2/TLR4 heterodimer of microglia via the MyD88 to activate the NF-κB, which further promote many inflammatory factors’ production, such as TNF-α, IL-6 etc. As SsnB could cut off the association of MyD88 with TLR4 and TLR2, so whether it could also act on the formation of TLR2/TLR4 heterodimer to protect against the inflammatory brain injury after ICH still need to be investigated.

Moreover, a clinical study has demonstrated that the upregulated expression of TLR2 and TLR4 correlated with poor outcome in patients with ICH [13], indicating that both TLR2 and TLR4 involved in the secondary inflammatory injury after ICH. And evidences showed that TLR2 can signal as a heterodimer with TLR1, TLR6 or TLR10 to activate the downstream signals [14, 15]. In addition, we recently demonstrated that the significantly increased expression of TLR2 can only be triggered by the Hb to cause brain inflammatory injury after ICH and the TLR2−/− mice exhibited more inflammatory injury compared with the WT, TLR6−/− and TLR1−/− mice following ICH [16].
Furthermore, more significant results were acquired by western blot, immunoprecipitation and immunoﬂuorescence technologies demonstrated that TLR2 forms a heterodimer with TLR4 mediated ICH-induced inflammatory injury, but there were no TLR1/TLR2, TLR1/TLR4 or TLR2/TLR6 heterodimerizations in both in vitro and in vivo models of ICH [16]; and the Hb, no other blood components, triggers the formation of TLR2/TLR4 heterodimer via MyD88, rather than TRIF to induce inflammatory injury in ICH; and the mutation of MyD88 Arg196 abolished the TLR2/TLR4 heterodimerization [16]. (Figure) This research results have more significances in the ICH-induced inflammatory injury, which not only further explain the upstream events that initiate inflammatory responses following ICH, but also provide a novel target-interfering with the assembly of TLR2/TLR4 heterodimer-for developing an effective treatment of ICH. And if we could intervene the formation of TLR2/TLR4 heterodimer after ICH, it may not only has an obvious therapeutic effect for ICH and also do not reduce the natural functions of TLR2 and TLR4, which in turn will not increase the secondary infection risk.

It is exciting to note that a previously research showed that SparstoloninB (SsnB), extracted from a Chinese herb Spaganium stoloniferum, was able to cut off the association of MyD88 with TLR4 and TLR2, and could selectively block the TLR2- and TLR4-mediated inflammatory signaling [17]. However, whether SsnB could act on the formation of TLR2/TLR4 heterodimer to protect against the inflammatory brain injury after ICH (Figure), and are there any compounds like SsnB have the same properties to inhibit the TLR2/TLR4 heterodimer mediated inflammatory injury still need to be investigated.

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Conflict of interest

The authors declare that there is no conflict of interest.

References