Janus Kinase Inhibition Ameliorates Cerebral Ischemic Injury and Neuroinflammation through Reducing NLRP3 Inflammasome Activation via JAK2/STAT3 Pathway Inhibition

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Research

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Abstract

Background

Evidence shows that inflammatory responses play multiphasic roles in stroke pathogenesis. Ruxolitinib (Rux), a selective oral JAK 1/2 inhibitor, is efficacious in COVID-19 by reducing inflammation via the JAK2/STAT3 pathway.

Methods

Here, we investigated whether JAK2 inhibition has neuroprotective effects against ischemic stroke (IS) in MCAO mice in vivo and in vitro oxygen-glucose deprivation/reoxygenation (OGD/R) model, and explored the potential molecular mechanisms. Rux was applied to MCAO mice. Immunofluorescence staining, RT-qPCR, and western blots were used to measure the expression of NLRP3 inflammation components and proinflammatory cytokines as well as JAK2/STAT3 pathway. Local STAT3 deficiency in brain tissue was established to investigate the interplay between NLRP3 and STAT3 signaling.

Results

Rux treatment obviously improved neurological scores, decreased the infarct size and ameliorated cerebral edema 3 days after stroke. In addition, immunofluorescence staining and western blots showed that Rux application inhibited the expression of NLRP3 inflammasome components, proteins related to the NLRP3 inflammasome and phosphorylated STAT3 (p-STAT3) in neurons. Furthermore, Rux administration inhibited the expression of proinflammatory cytokines, including TNF-α, IFN-γ, HMBG1, IL-1β, IL-2, and IL-6 in middle cerebral artery occlusion (MCAO) model mice, suggesting that Rux may alleviate IS injury by inhibiting proinflammatory reactions via JAK2/STAT3 signaling pathway regulation. Local STAT3 deficiency decreased histone H3 and H4 acetylation on the NLRP3 promoter and the NLRP3 inflammasome component expression, indicating that the NLRP3 inflammasome may be directly regulated by STAT3 signaling. Finally, the effect of Rux on the NLRP3 inflammasome was further assessed in a HT22 cell OGD/R model in vitro. Rux application markedly suppressed lipopolysaccharide (LPS)-induced NLRP3 inflammasome secretion and JAK2/STAT3 pathway activation in vitro the in OGD/R HT22 cell model.

Conclusion

JAK2 inhibition by Rux in MCAO mice decreased STAT3 phosphorylation, thus inhibiting downstream proinflammatory cytokines and H3 and H4 acetylation on the NLRP3 promoter, resulting in downregulation of NLRP3 inflammasome component expression.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.
### Figures

**Figure 1**

Rux induced infarct size, neurological deficits and cerebral edema in mice after MCAO. (A) Representative TTC-stained slices showing infarction in vehicle- and Rux-treated mice. (B) Quantitative analysis of the infarct size was presented as a percentage of the contralateral hemisphere size 3 days after stroke. (C) Neurological scores were determined 3 days after MCAO. (D) Representative western blots showing that Rux application reduced AQP4 expression. (E) Quantification of AQP4 expression. (F) Quantification of water content. Mean ± SD. n = 5 in each group. aP < 0.05 versus the vehicle group; bP < 0.05 versus the 30 mg/kg Rux group; cP < 0.05 versus the 60 mg/kg Rux group.
Administration of Rux decreased the number of NLRP3+ and GFAP+ cells in the ischemic penumbra. (A) Representative images of GFAP and NLRP3 immunostaining in the penumbra zone sham-, vehicle-, Rux- and MCC950-treated mice 3 days after IS. (B, C) Statistical analysis of cell counting of NLRP3+ and GFAP+ cells. (D) Quantification results of double-positive cells in the peri-ischemic region. Mean ± SD. n = 5. aP < 0.01 versus sham; bP < 0.01 versus vehicle. Scale bar: 50 μm.
Figure 3

Rux treatment decreased the number of NLRP3+ and CD68+ cells in the ischemic penumbra. (A) Representative images of NLRP3 and CD68 immunostaining in the ischemic penumbra in sham-, vehicle-, Rux- and MCC950-treated mice 3 days after stroke. (B, C) Statistical analysis of NLRP3+ and CD68+ cells. (D) Quantification of double-positive cells in the peri-ischemic region. Mean ± SD. n = 5. aP < 0.01 versus sham; bP < 0.01 versus vehicle. Scale bar: 50 μm.
Figure 4

Rux treatment reduced the number of NLRP3+ cells and increased the number of NeuN+ cells in ischemic penumbra 3 days after MCAO. (A) Representative immunostainings of NLRP3 and NeuN in the penumbra zone in sham-, vehicle-, Rux- and MCC950-treated mice. (B,C) Statistical analysis of NLRP3+ and CD68+ cells. (D) Quantification results of double-positive cells in the peri-ischemic region. Mean ± SD. n = 5. aP < 0.01 versus sham; bP < 0.01 versus vehicle; cP < 0.05 versus Rux. Scale bar: 50 μm.
Figure 5

Rux treatment suppressed the NLRP3 inflammasome associated proteins. (A) Representative western blots showing that administration of Rux reduced the expression of NLRP3, ASC, CL-caspase-1, IL-18 and IL-1β in the peri-ischemic region 3 days after MCAO. (B-F) Quantification results of NLRP3, ASC, CL-caspase-1, IL-18 and IL-1β protein levels in the ischemic cortex. Mean ± SD. n = 5/group. aP < 0.01 versus sham; bP < 0.01 versus vehicle; cP < 0.05 versus Rux.
Figure 6

Rux treatment reduced the mRNA expression of proinflammatory cytokines 3 days after MCAO. (A-F) mRNA expression of IL-1β, IL-2, IL-6, HMGB1, TNF-α, and IFN-γ. (G,H) mRNA expression of IL-4 and IL-10. The data are shown as the fold change compared with the sham. n = 5. aP < 0.001 versus sham; bP < 0.01 versus vehicle; cP < 0.05 versus Rux.

![Western Blot Image]

Figure 7

The administration of Rux inhibited the activation of p-JAK2 and p-STAT3 3 days after MCAO. (A) Representative western blots showing that Rux inhibited p-JAK2/p-STAT3 expression in the peri-ischemic region 3 days after MCAO. (B, C) Quantification of p-JAK2/p-STAT3 expression in ischemic penumbra. Mean ± SD, n = 5/group. aP < 0.01 versus sham; bP < 0.05 versus vehicle; cP < 0.05 versus Rux.
Local STAT3 deficiency decreased histone H3 and H4 acetylation an NLRP3 expression. (A) Representative immunostainings of NLRP3 and p-STAT3 in penumbra of the ischemic cortex 3 days after MCAO. (B) Representative western blots showing that Rux application restrained p-JAK2 and p-STAT3 expression in the peri-ischemic region 3 days after MCAO. (C-G) Quantification of STAT3, p-STAT3, NLRP3, Ac-H3 and Ac-H4 levels on the NLRP3 promotor in the ischemic penumbra. Mean ± SD. n = 5/group. aP < 0.001 versus sham; bP < 0.05 versus lenti-GFP. Scale bar: 50 μm.
Figure 9

Administration of Rux reduced NLRP3 inflammasome activation in HT22 cells after OGD/R. (A) Representative western blots showing NLRP3, ASC, CL-caspase-1, IL-1β and IL-18 in protein level. (B-F) Quantification results of NLRP3, ASC, CL-caspase-1, IL-1β and IL-18 expression at the protein level. Mean ± SD. n = 5/group. aP < 0.01 versus sham; bP < 0.05 versus OGD/R + DMSO; cP < 0.05 versus OGD/R + LPS.

Figure 10

Rux treatment inhibited the LPS-induced JAK2/STAT3 activation in HT22 cells after OGD/R. (A) Representative western blots showing that Rux administration downregulated p-JAK2/p-STAT3
expression in HT22 cells after OGD/R. (B, C) Quantification of p-JAK2 and p-STAT3 presented as the fold change. Mean ± SD. n = 5/group. aP < 0.01 versus sham; bP < 0.05 versus OGD/R + DMSO; cP < 0.01 versus OGD/R + LPS.

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