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THE ROLE OF CXCR3 (CHEMOKINE (C-X-C MOTIF) RECEPTOR 3) IN INTRACEREBRAL HEMORRHAGE

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Background

- Intracerebral haemorrhage (ICH) is associated with high mortality and morbidity rates.
- There is no treatment that effectively improves prognosis.
- This preclinical study aims at deciphering the role of C-X-C Motif Chemokine Receptor 3 (CXCR3), a potential therapeutic target, in ICH.
- CXCR3 is a receptor for multiple ligands of different affinities, namely CXCL9, CXCL10, CXCL11 and CXCL4. CXCL10 has been linked to a worse ICH outcome in a recent clinical study.
- Literature has shown that CXCR3 contributes to the pathogenesis of atherosclerosis. The circulating levels of its ligands are significantly upregulated in patients with hypertension, who are susceptible to the development of ICH. Yet, its involvement in ICH is unknown.
- The hypothesis of this study is that the inhibition of CXCR3 improves outcomes of experimental ICH in mice.

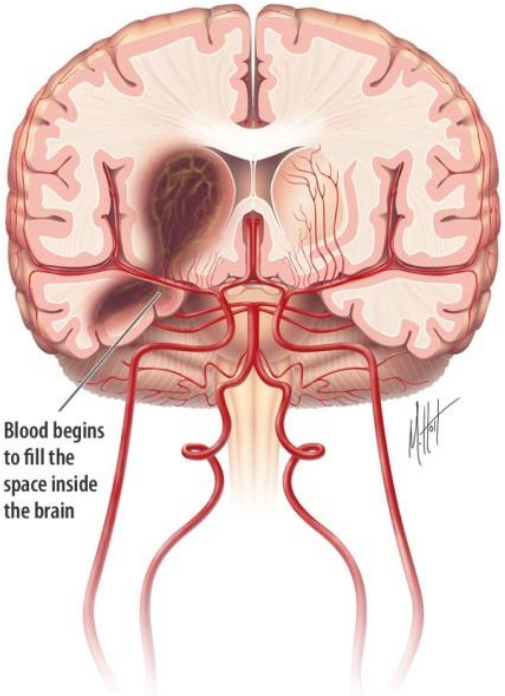


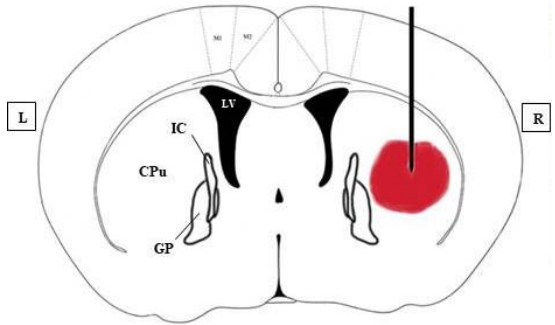
Image adapted from http://www.strokecenter.org/patients/about-stroke/intracerebral-hemorrhage/

Methodology

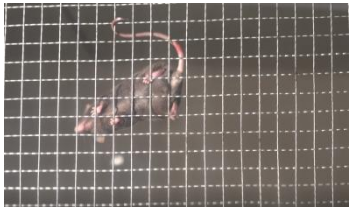
- **ICH induction**: 12 to 14-week-old adult male wild type (WT) C57BL/6 mice and CXCR3-KO (Cxcr3^{tm1Wwh}) mice were used. 0.5µL of 0.08U/µL type IV bacterial collagenase in normal saline was injected slowly at 0.175 µL /min into the right striatum 3.5 mm deep to the dura using a 26-gauge Hamilton syringe (10µL) through a burr hole with a diameter of 0.15mm located 0.2 mm anterior to the bregma and 2 mm lateral to the midline on the right side of the skull. Sham operation only involved needle insertion.

- **Behavioural testing**: Modified neurological severity score (mNSS) was used for determining the successful establishment of model and also for testing several motor functions at baseline and Day 1 post-ICH. Other behavioural tests including cylinder test, rotarod test and grid walking test were performed at baseline, Day 3, Day 7, Day 14 and Day 28 post-ICH.

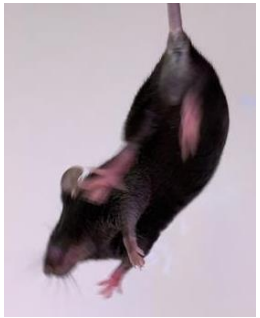
- **Statistical analysis:** Mixed-effect model was used for the analysis of behavioural data.



Collagenase mice model



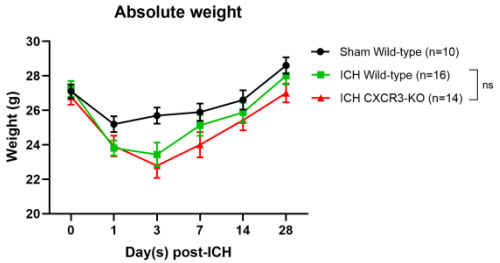
Grid walking test

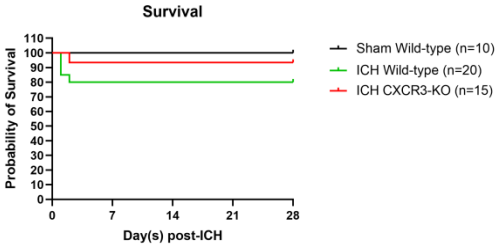


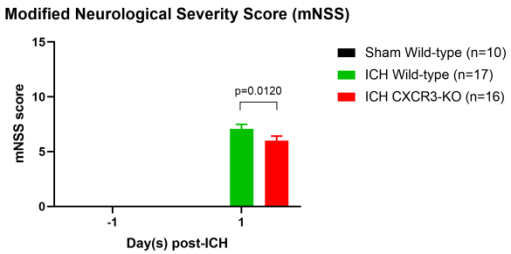
mNSS

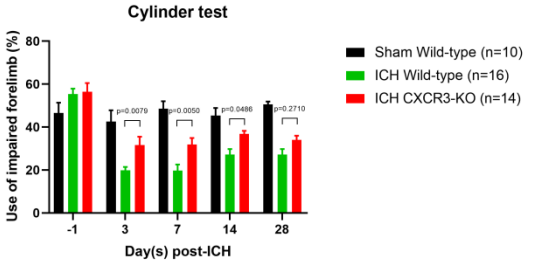
Results

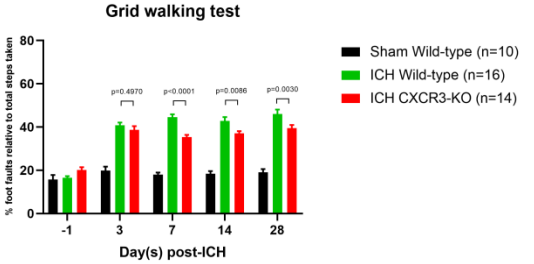
- The CXCR3-KO ICH group had a significantly lower mNSS score at Day 1, implying a milder degree of injury compared with that of Wild-type ICH group.
- The CXCR3-KO ICH group had better motor performance than the Wild-type ICH group
  - At D3, D7 and D14 for cylinder test
  - At D7, D14 and D28 for grid walking test
  - At D3 and D7 for rotarod test
- There were no significant differences in weight and survival between the ICH groups.

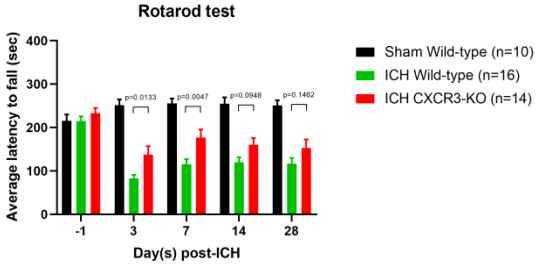






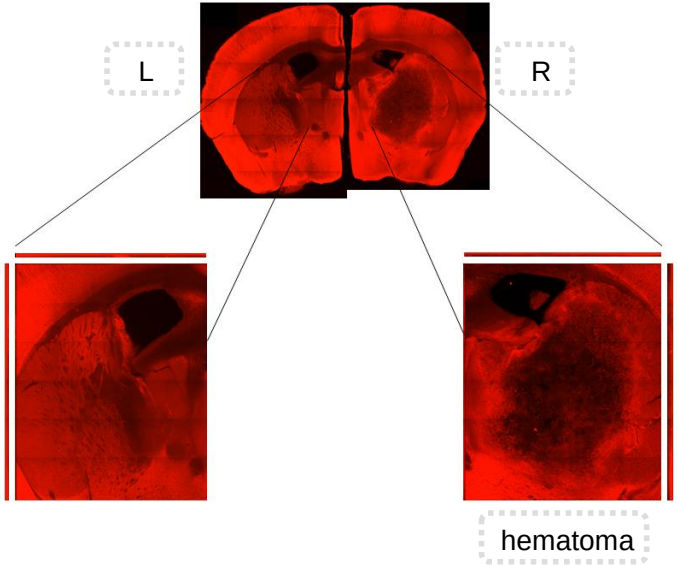






Discussion

- The behavioural test results showed that in vivo inhibition of CXCR3 was associated with better behavioural outcomes after ICH over the observed period although, in this preclinical model, the statistical significance was lost at later time points.
- CXCR3 inhibition may not provide survival benefit during the acute phase after ICH.
- The mechanism through which CXCR3 contributes to the pathogenesis of ICH is under investigation.
- For better preservation of the anatomical structures and easier visualization of white matter tract organization after ICH, we are currently working on tissue clearing plus immunofluorescence staining. The idea is that whole brain samples can be cleared by a specific reagent that also preserves myelin sheath. Thicker tissue blocks (at least 1mm thick), rather than the usual brain sections (around 10μm thick), can be visualized using confocal microscopy. This technique can benefit stroke research by revealing the pathogenic process in a much larger region.



Example: Day 3 post-ICH, Immunofluorescence staining of Myelin Basic Protein (MBP) using LSM 800 confocal microscope at 10x and 20x magnification.

Conclusion

- C-X-C Motif Chemokine Receptor 3 (CXCR3) may take part in the pathogenesis of ICH.
- Inhibition of CXCR3 could potentially be a novel therapeutic option.