Vitamin D_3 as a possible therapeutic agent for promoting remyelination after intracerebral haemorrhage

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Background

- Haemorrhagic stroke
  - Intracerebral haemorrhage (ICH) accounts for 20-30% of stroke incidence in the Asian population. In addition to its high mortality rate, ICH is associated with poor long-term functional recovery among survivors, which may be due to massive neuronal death and demyelination.
  - Management of ICH mainly targets disease mechanisms in the acute phase, such as the mass effect of the haematoma, tissue response to the haematoma and clot components. However, research on regenerative treatment strategies in the subacute phase of ICH remain sparse.
- The role of vitamin D in CNS disorders
  - Various studies have demonstrated the effects of vitamin D in improving functional outcomes after injuries and diseases of the CNS, such as multiple sclerosis. It is noted to have multiple effects on different cell types, including neuroprotection and stimulation of endogenous regeneration.

Methodology

- Mouse collagenase ICH model
  - Haemorrhagic stroke was induced in the right corpus striatum.
  - A burr hole with a diameter of 0.6 mm with the following coordinates was drilled:
    - X (mediolateral) -0.2 (2mm to the right of the bregma)
    - Y (anteroposterior) + 0.02 (0.2 mm anterior to the bregma)
  - 0.55 μl of type IV bacterial (Clostridia) collagenase infused at 0.175 μl/min using a 30-gauge needle with its tip 3.5 mm below the dural surface
- Experimental arms (Wild type male C57BL/6N mice, 10 wks old)
  - Treatment group: Daily oral administration of 2μl of vitamin D_3 diluted in 8 μl of cooking oil
  - Control group: Daily oral administration of 10μl of cooking oil
  - Sham group: Daily oral administration of 10μl of cooking oil
- Experimental timeline:
  - Baseline → induction of ICH → oral vitamin D3 supplementation daily up to experimental endpoint → motor tests performed at experimental timepoints + tissue harvest on the same day for protein and histological analysis
  - Experimental endpoints: D7, D14, D21 post-ICH
- Assessments
  - Protein analysis: Western blotting
  - Histology: immunofluorescence staining on brain frozen sections, transmission electron microscope imaging of the cervical spinal cord dorsal corticospinal tract
  - Behavioural motor assessments: Modified Neurological Severity Scores (mNSS), forelimb asymmetry test (cylinder test), accelerated rotarod test

Results

The results are preliminary as the study is still ongoing.

GraphPad Prism (version 8) software was used for statistical analysis. The data were shown as mean ± standard error of mean.

Figures (1) and (2): Forelimb asymmetry (cylinder) test and mNSS results both demonstrate an association between improved recovery of motor function and supraphysiological levels of vitamin D_3 supplementation, the effects of which were observable as early as D7.

Figures (3) and (4): Analysis of cell lysates from the the ipsilesional right striatum using GAPDH as the housekeeping protein indicate an upregulation of A2B5, a marker of OPCs, upon VD_3 supplementation at D7 and 14 post-ICH (fig.3). Vitamin D receptor upregulation was also observed at D7 and D 14 post-ICH in both vehicle and vitamin D-treated mice, the latter of which led to a more significant increase in VDR (fig.4).

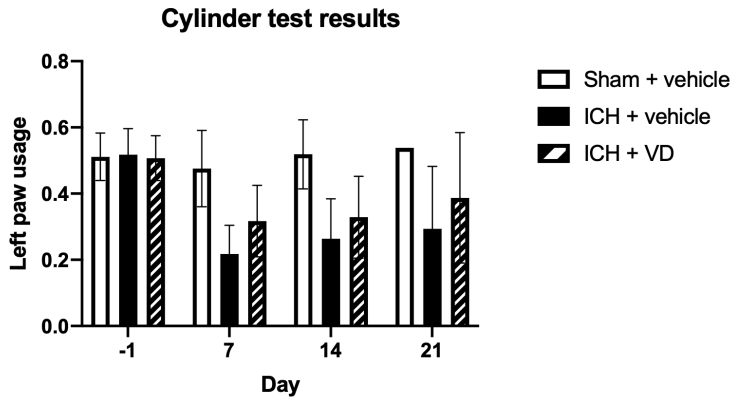

Figure (1)

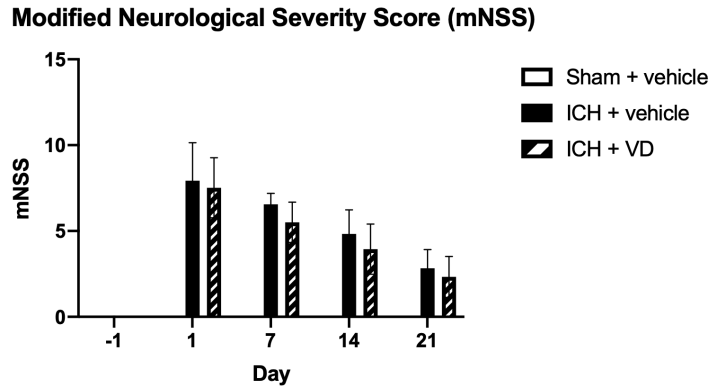

Figure (2)

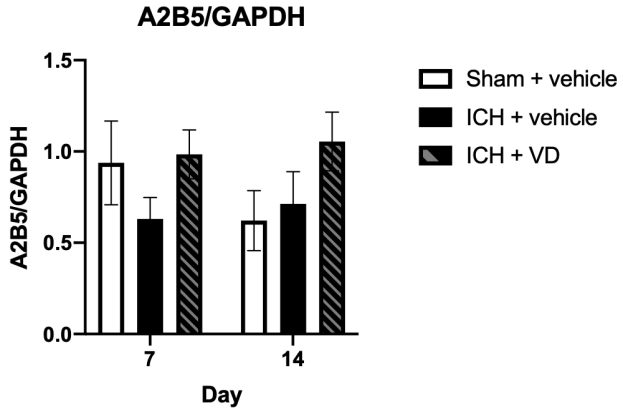

Figure (3)

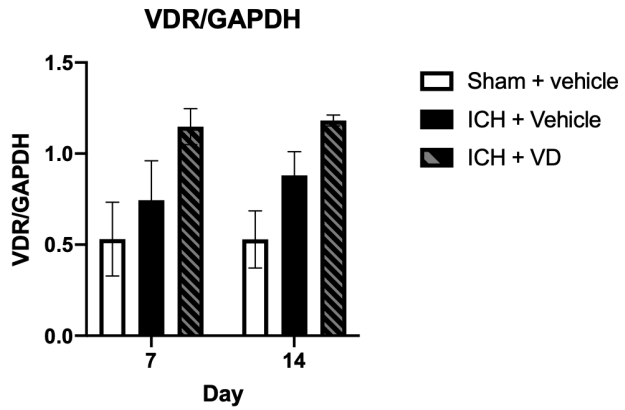

Figure (4)

Discussions

- Taking the poor prognosis of ICH and long-term functional deficits among survivors into account, it should be noted that treatment for ICH should not be limited to management in the acute phase. Regenerative treatment strategies should be studied in greater detail in addition to investigations on the pathogenesis of secondary brain injuries and strategies to limit injury mechanisms. Such may further enhance post-stroke recovery, especially when the hostile microenvironment in the lesioned site proves unsuitable for intervention in the acute phase.
- A previous study on oligodendrocyte-lineage cell behaviour in ICH has demonstrated an increase in oligodendrocyte precursor cell and oligodendrocyte population in the perihaematoma region in the subacute phase of ICH, suggesting that they might contribute to tissue regeneration and repair through remyelination. (1) However, there has been no evidence on whether and how such oligodendrocyte-lineage cells integrate into existing circuits to promote functional recovery. No concrete evidence of remyelination in the chronic stage of ICH currently exist.
- This is the first study aiming to demonstrate post-ICH demyelination and subsequent remyelination in through observing the microstructure of the spinal cord corticospinal tract by electron microscope imaging. The spinal cord instead of the brain peri-haematoma region was chosen for the ease of imaging and localization of the corticospinal tract, the damage to which contributes to the motor deficits observed in mice with ICH. The results obtained from such imaging studies will allow for quantitative assessment of the myelin status in the corticospinal tract, lesions in which are more directly correlated with motor performance as demonstrated by behavioural assessments.
- While it has been demonstrated that vitamin D serves as a differentiation-promoting agent to improve CNS injury outcomes, such as on OPCs in a rat spinal cord injury model, the effects of vitamin D on OPCs in an ICH disease model has not been investigated. This study also aims to investigate the potential therapeutic effects of vitamin D supplementation through different downstream pathways.

(1) Joseph, M. J. E., Caliaperumal, J., & Schlichter, L. C. (2016). After Intracerebral Hemorrhage, Oligodendrocyte Precursors Proliferate and Differentiate Inside White-Matter Tracts in the Rat Striatum. *Translational Stroke Research*, *7*(3), 192–208. https://doi.org/10.1007/s12975-015-0445-3

Conclusion

- The preliminary results from this study support the hypothesis that vitamin D_3 supplementation is associated with hastened long-term functional recovery after haemorrhagic stroke in an ICH mouse model.
- Such improvements may be partly be associated with enhanced remyelination due the action of vitamin D_3 on promoting the differentiation of oligodendrocyte precursor cells into oligodendrocytes. Further assessments will be performed to investigate the potential downstream pathways activated by vitamin D_3.
- Pending results:
  - EM images of the spinal cord CST
  - Western blot results comparing the levels of various proteins at D7, D14 and D21 post-ICH
  - Immunofluorescence staining for localisasing of OPCs in the perihaematoma region and surrounding striatum
  - Effects of ICH on the contralestional striatum and the effects of vitamin D_3 on the normal brain