SPLENECTOMY IMPROVES FUNCTIONAL OUTCOME OF EXPERIMENTAL INTRACEREBRAL HAEMORRHAGE (ICH) IN MOUSE MODELS THROUGH INCREASED HAEMATOMA RESORPTION

Chun Hin LEUNG[1], Hei Tung SHEK[1], Jiaxin LIU[1], Gilberto Ka-Kit LEUNG[1]

[1]Division of Neurosurgery, Department of Surgery, School of Clinical Medicine, LKS Faculty of Medicine, the University of Hong Kong, Queen Mary Hospital, Hong Kong

INTRODUCTION

Intracerebral hemorrhage refers to bleeding within the brain parenchyma, and it is associated with high mortality and currently lacks definitive treatment. It is clear that there will be neutrophil and other immune cells infiltrating the brain after ICH, proving peripheral immune system plays an important role contributing to various processes such as neuroinflammation, hematoma expansion and resorption. Nevertheless, the spleen as a large pool of immune cells in the peripheral immune system has not been studied extensively in the context of ICH. Therefore, the present study pioneers to evaluate role of splenectomy in speeding up hematoma resorption and recovery.

METHODS

C57BL/6 male mice of 8-12 weeks and around 25g were housed in temperature and humidity controlled facility with 12 hour light/dark cycle. Food and water were provided ad libitum. Mouse were divided into 2 groups: ICH mice with splenectomy and ICH mice with laparotomy only.

Splenectomy were performed 2 weeks prior to ICH to minimise effect of surgery. 2cm midline incision was made to the abdominal skin and peritoneum. The spleen was removed by cauterising its vessels at the hilum. As for sham splenectomy, only laparotomy was performed with the spleen intact.

ICH was induced by intra-striatal injection of type IV collagenase (2mm to the right, and 0.2 mm anterior to bregma, and 3.5mm deep).

The neurological deficits post-ICH were measured using modified Neurological Severity Score (mNSS), rotarod test and cylinder test.

To assess hematoma volume, mouse were sacrificed on day 1 and 3, and the brains were sectioned into 1mm slices. Slices were photographed and analysed using photoshop and Image J to measure hematoma area. The area are multiplied by thickness of each slice and summed up to obtain hematoma volume. The hemoglobin concentration was then evaluated using Drabkin's solution based colorimetric method using 520nm filter.

Independent T test was used to evaluate differences in hematoma volume and hemoglobin concentration between 2 groups.

RESULTS AND DISCUSSION

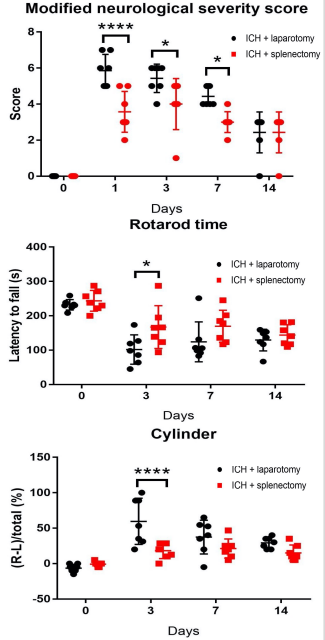

Figure 1a – 1c (top to bottom): significant reduction in mNSS on day 1,3,7 in spx group. Significant higher rotarod time on day3 in Spx group. Significant reduction in laterality index on day 3 in Spx group

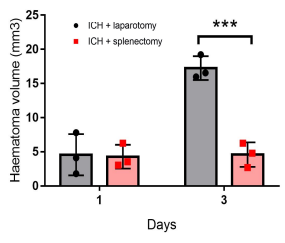

Figure 2: Hematoma volumes of mouse brain on day 1 and 3 after ICH induction. Hematoma volume is significantly smaller in splenectomy group on day3 (p=0.0002, n=8)

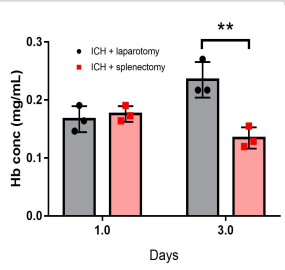

Figure 3: Hemoglobin concentrations of right hemispheres (with hematoma) on day 1 and 3. The hemoglobin concentrations are significantly lower in splenectomy group on day3 (p=0.0011, n=8)

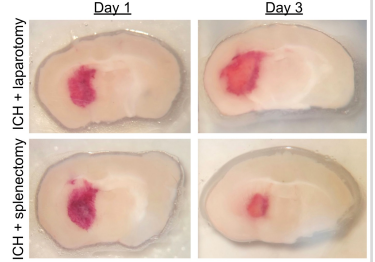

Figure 4: Hematoma sizes of mouse brain sections on day 1 and 3

Splenectomy was associated with improved neurological outcomes on day3. this can be attributed to the significant reduction in hematoma volume on day3 in splenectomy group compared to control group. It is confirmed by a significant reduction in hemoglobin concentration on day 3. Nevertheless, both hematoma volume and hemoglobin concentration were similar between splenectomy and control group. It is worth noting the hematoma volume decreases from day1 to 3 in splenectomy group, while that of control group increases from day1 to 3. This suggest removal of spleen speeds up hematoma resorption and slow down hematoma expansions, possibly via reduction in immune cells infiltration, further research will be carried out to evaluate the underlying causes.

CONCLUSION

Splenectomy significantly reduces hematoma volumes, proving the vital role of spleen in hematoma expansion in ICH.

REFERENCES

1. LEE S.T. et al. (2008). Anti-inflammatory mechanisms of intravascular neural stem cells transplantation in hemorrhagic stroke. Brain 131 (Pt3), 616-629. DOI: 10.1093/brain/awm306

2. Dotson et al. (2015). Splenectomy reduces infarct volume and neuroinflammation in male but not female mice in experimental stroke. Journal of Neuroimmunology 278, 289-298DOI:https://doi.org/10.1016/j.jneuroim.2014.11.020

3. Chauhan et al. (2018). Splenectomy protects aged mice from injury after experimental stroke. Neurobiol aging 61, 102-111. DOI: 10.1016/j.neurobiolaging.2017.09.022

Contact information

LEUNG Chun Hin (MRes[Med] student): lch1512@connect.hku.hk

SHEK Hei Tung (Mres[Med] student): Pinky12@connect.hku.hk

Professor Gilberto Leung: gilberto@hku.hk

香港大學外科學系 HKU SURGERY