Copper Homeostasis and Cuproptosis in intracerebral haemorrhage (ICH)

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INTRODUCTION

Intracerebral haemorrhage (ICH) is a subtype of stroke, characterised by bleeding into brain parenchyma. In 2022, studies discovered a novel mechanism of cell death, cuproptosis, referring to copper-induced cell death. In ICH, disruption of blood-brain barrier followed by infiltration of copper from plasma into brain, results in copper overload and cuproptosis. Oligomerization of Dihydrolipoamide S-Acetyltransferase (DLAT) has been identified as a hallmark of cuprotosis (Tsvetkov et al., 2022). We aim to study whether ICH would induce cuproptosis as a result of DLAT accumulation.

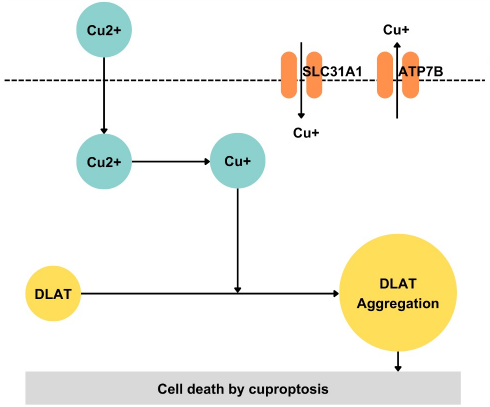

***Figure 1**: Mechanism of cuproptosis. Copper is transported into cells via CTR1 which is encoded by SLC31A1 and exported via ATP7B. Imported copper binds to lipoylated DLAT, results in oligomerization of DLAT and subsequently proteotoxic stress, leading to cell death.*

METHODS

ICH was induced in C57BL/6 mice by collagenase injection into right striatum of brain, while sham surgery was performed as control without collagenase injection. Copper levels in hematoma and peri-hematoma region were measured using colorimetric copper assay kit. Western blot was performed to detect DLAT oligomer. Expression level of copper transporter SLC31A1 was measured by quantitative polymerase chain reaction (qPCR).

RESULTS

1. Copper level in Sham and ICH

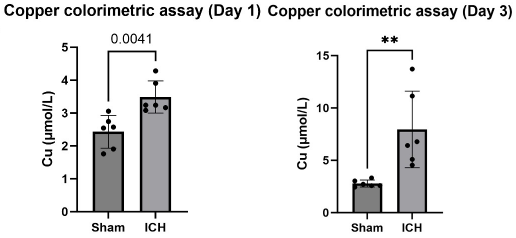

***Figure 2a-b**: Copper level in Sham and ICH on day 1 and day 3. Samples were obtained from the right striatum of sham model, ICH day 1 and ICH day 3 mice model.*

Copper level is higher in ICH (day 1, p = 0.0041; day 3) than Sham in the ipsilateral striatum. These data suggest that there is an infiltration of copper from plasma into brain in ICH, which may result in copper overload.

2. DLAT oligomer level in Sham and ICH

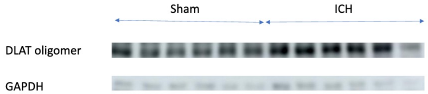

***Figure 3a**: Western blot results of sham models and mouse models on day 14.*

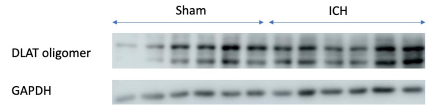

***Figure 3b**: Western blot results of sham models and mouse models on day 7.*

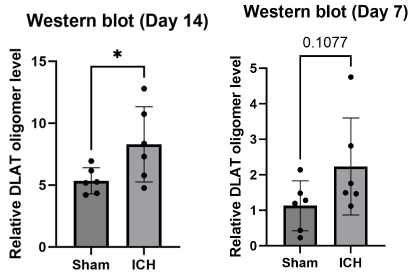

***Figure 3c-d**: Western blot quantification of sham models and mouse models on day 14 and day 7.*

Lysate samples from ICH (day 14; day 7) mice showed a higher level of DLAT oligomer than those samples from sham mice. DLAT oligomer is a hallmark of cuproptosis; the western blot result shows that the level of cuproptosis is higher in ICH than sham mice. These data suggest that cuproptosis contributes to cell death in ICH.

3. SLC31A1 expression level

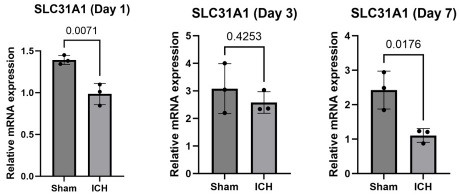

***Figure 4a-c**: qPCR results of sham models and mouse models on day 1,3 and 7.*

SLC31A1 expression is lower on ICH day 1 (p = 0.0071), day 3 and day 7 (p = 0.0176) than that in sham group. It was shown that elevated intracellular copper level stimulates downregulation and endocytosis of CTR1, a copper transporter encoded by SLC31A1 gene (Gaggelli et al., 2006). These data suggest that there is a downregulation of CTR1 in ICH to protect cells from toxic effect of excess copper in blood, which may imply the presence of instrinsic mechanism in protecting cell from cuproptosis.

CONCLUSION

Copper overload is observed in ICH, suggesting that cuproptosis plays a role in causing cell death in ICH through inducing DLAT aggregation.

REFERENCES

Tsvetkov et al. (2022). DOI: 10.1126/science.abf0529.

Gaggelli et al. (2006). DOI: 10.1074/jbc.M807909200.