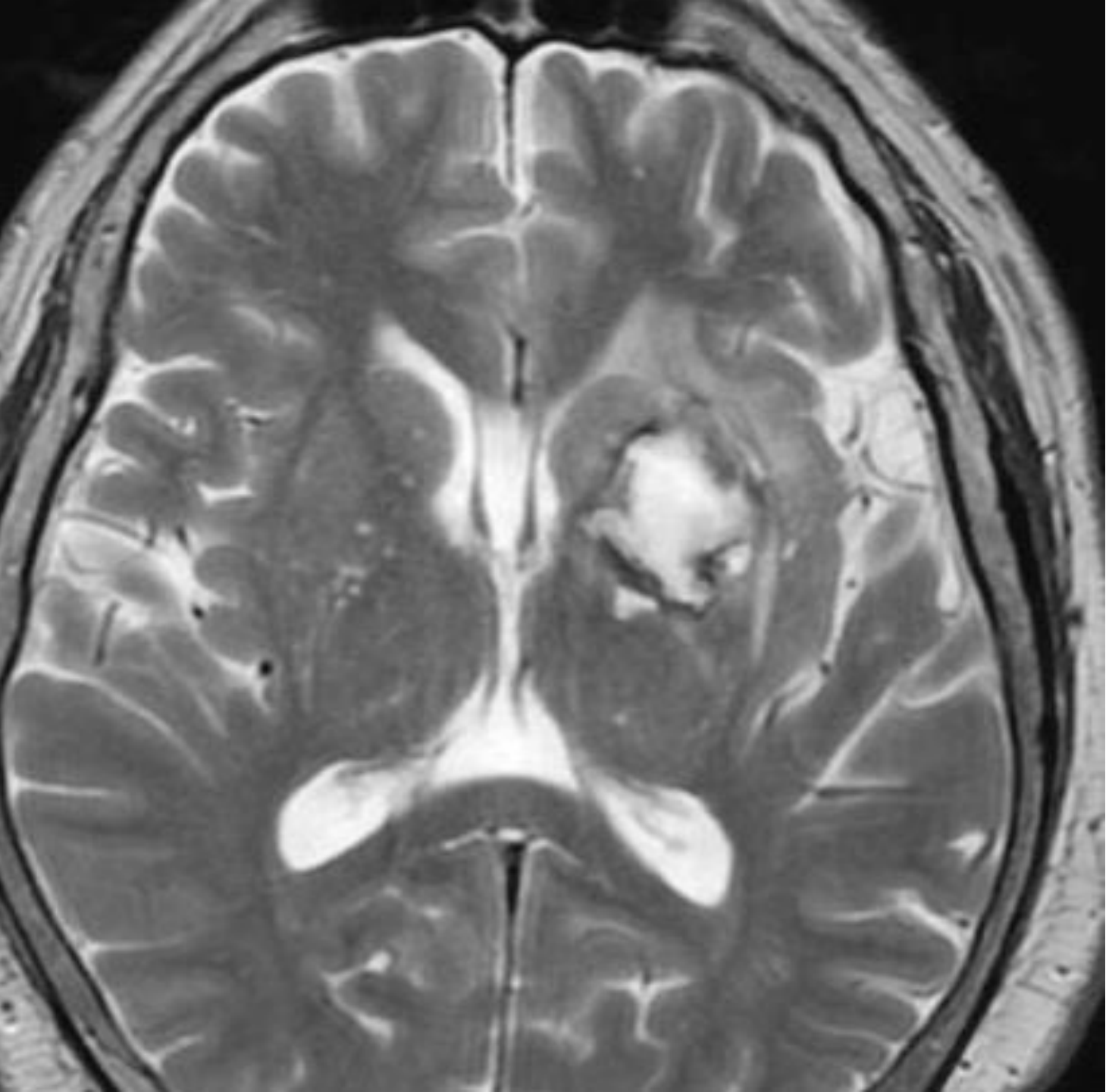

LKS Faculty of Medicine
The University of Hong Kong
香港大學李嘉誠醫學院

Cerebral mast cells as a potential therapeutic target for intracerebral hemorrhage

CHAN Andrian Anderson

CHENG Stephen

LEUNG Gilberto Ka Kit

Division of Neurosurgery, Department of Surgery, Queen Mary Hospital, Hong Kong

Background

Intracerebral hemorrhage (ICH) is the most common subtype of hemorrhagic stroke in the world, and in Hong Kong accounts for up to 35% of all strokes. Approximately 50% of patients die within 30 days of the stroke event, and only a minority of those who survive are expected to be functionally independent. Because of the current lack of treatment regimens that reliably improve patient outcome, there is a pertinent necessity for research into this devastating stroke.

Mast cells are classically known for their role in allergic pathology, but in recent years have also been recognized to play a notable role in the pathogenesis of multiple forms of brain injury [1]. New studies have attempted to modulate mast cell activity to reduce the damage caused by experimental ICH in a variety of models.

Vitamin D3 (1, 25(OH) 2D3), also known as cholecalciferol, is a steroid hormone synthesized in human skin from 7-dehydrocholesterol by UV light, and is primarily metabolized in the liver and then in the kidney into calcitriol [2]. Calcitriol is the most biologically active metabolite of vitamin D and an FDA-approved molecule whose neuroprotective and neurotrophic actions are being increasingly recognized [3].

Crucially, the incidence of atopic diseases has been correlated in international and preclinical studies with a deficiency of vitamin D [4-5].

Objectives

- To elucidate mechanisms by with vitamin D may exert its neuroprotective effect, if any, in ICH
- To explore the contribution of cerebral mast cells in the formation of ICH pathology

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Methodology

Diet induction

Vitamin D deficient purified rodent diet containing alcohol-extracted casein, designed to contain 0.47% calcium and 0.3% phosphorus, was fed to the mice for 1 month prior to induction of ICH. Normal mice were fed regular chow throughout.

Experimental ICH

Male 8 – 12 weeks old C57BL/N mice were stereotactically injected with collagenase into the striatum to induce hemorrhage. At predetermined timepoints the mice were sacrificed; trans-cardiac perfusion was performed to harvest tissue.

Morphological study

After trans-cardiac perfusion, mice brains were harvested and sectioned in 1mm intervals. Hematoma volume was calculated; sections were photographed for reference.

Protein expression

Expression of mast cell tryptase and chymase was detected with western blotting technique; protein band strength was quantified with ImageJ software and data was analyzed with Prism.

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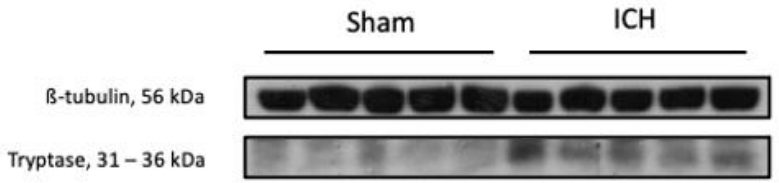
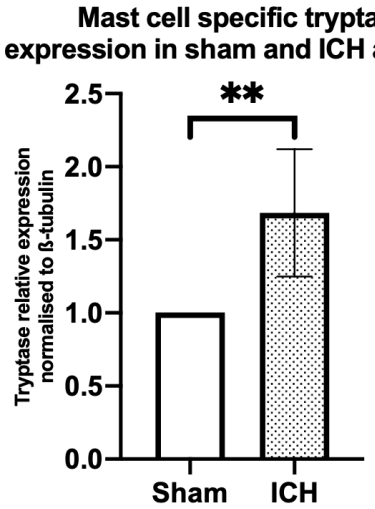
Results (1). Mast cell-specific proteases showed increased expression in experimental ICH

1.1





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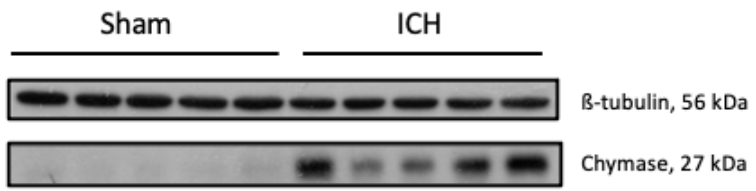

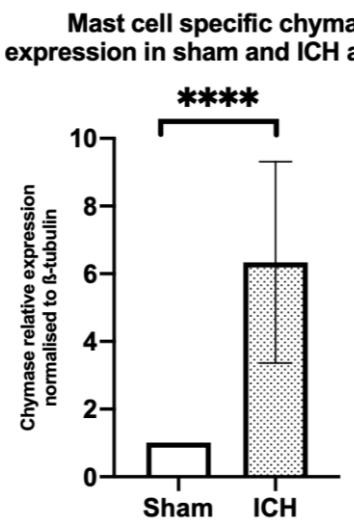

Figure 1. Western blotting analysis demonstrates increased expression of tryptase and chymase in ICH mice (n = 5) compared to mice that underwent sham surgery (n = 5) at 24h post-ICH.

Figure 1.1 Tryptase expression increased in ICH mice compared to sham group, **, $p \leq 0.01$.

Figure 1.2 Chymase expression increased in ICH mice compared to sham group, ****, $p \leq 0.001$

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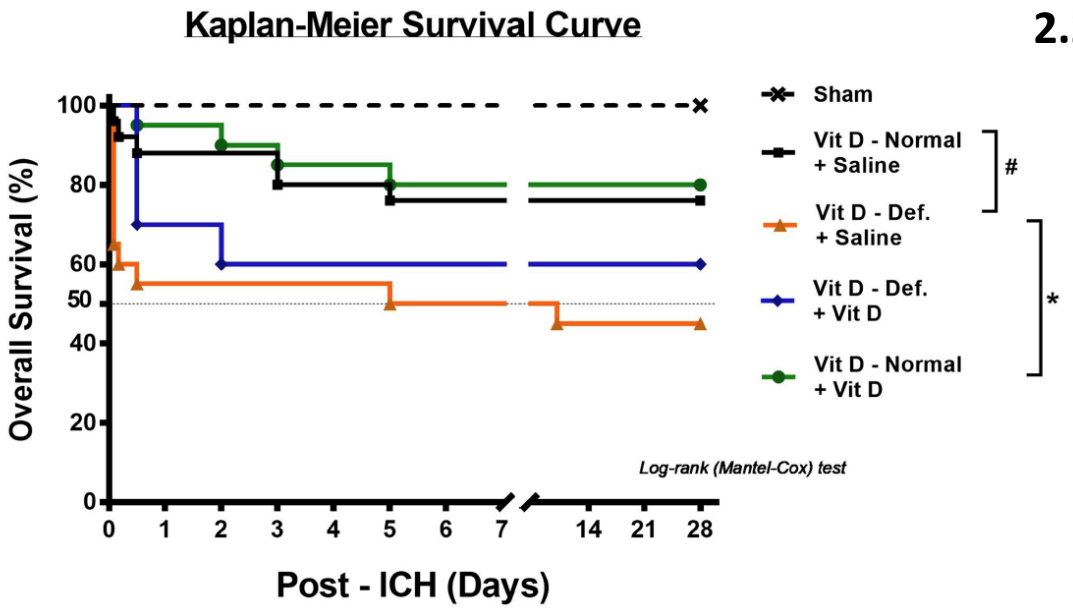
Results (2). Vitamin D deficient mice had worse outcome after experimental ICH

2.1



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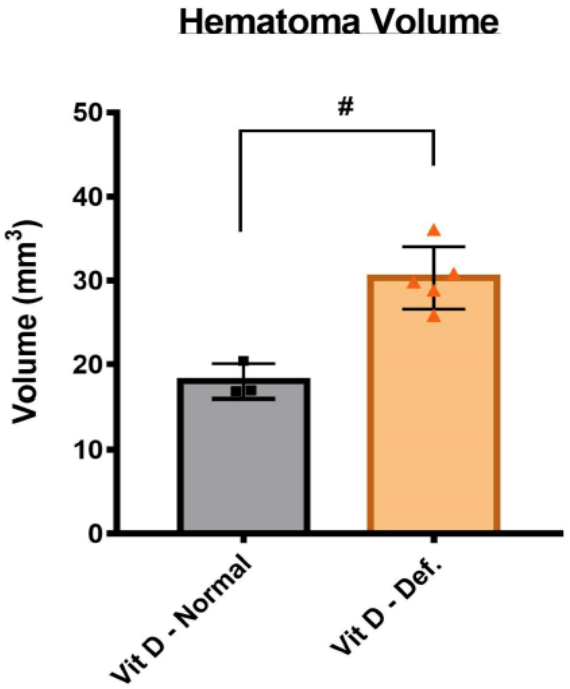

2.3

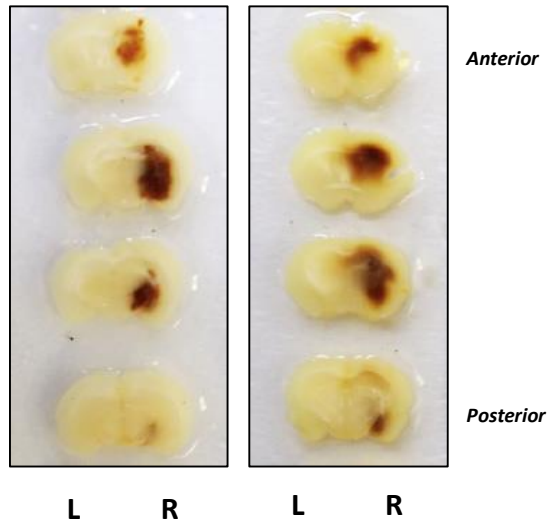

Figure 2.1 Vitamin D-deficient mice demonstrated worse survival in all groups, independent of treatment type (Orange: saline vehicle control; Blue: vitamin D supplemented). Mice with normal vitamin D levels had better survival regardless of treatment group. Vitamin D/Normal + saline v. vitamin D/Deficient + saline, #, $p \leq 0.05$).

Figure 2.2 Hematoma volume of untreated Vit D-deficient mice was 12% greater than hematoma volume in untreated, vit D-normal mice (#, $p \leq 0.05$).

Figure 2.3 1mm thick brain section showing larger hematoma volume in deficient mice versus normal diet mice, 24h post-ICH.

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Discussion and conclusion

Discussion. Our results show evidence of mast cell activation upon ICH in agreement with previous studies. Tryptase and chymase are mast cell-specific proteases that account for up to 25% of the protein content in MCs. It is possible that these proteases, upon rapid activation and degranulation of MCs, exacerbate the degradation of the blood brain barrier, hence driving the phenomenon of hematoma expansion. Vitamin D has been shown in numerous other studies to have neurotrophic and neuroprotective actions. Our results are the first to show that mice in a vitamin D-deficient state experienced worse outcome upon experimental ICH: they had larger hematoma volume and worse survival. Liu et al. 2017 found that mast cell lines were dependent on the presence of vitamin D to remain stable and granulated. Mechanistically, this might be due to an inhibition of its signal transduction cascades by vitamin D receptors complexing with Lyn tyrosine kinase to prevent downstream expression of NF-kB, and eventual degranulation [6].

Conclusion. The mechanistic study of vitamin D's neuroprotective role in ICH with relation to modulating mast cell activation shows promise and deserves greater attention. A deeper understanding of this potential acute therapeutic mechanism may translate into wider clinical application of a cheaply and easily accessible drug.

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