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Research report

Mouse model of intracerebellar haemorrhage

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HIGHLIGHTS

- Established mouse intracerebellar hemarrohage animal model.
- Analysis of motor deficits using open field and ataxia test at different time points.
- Perihematoma apoptotic cell death occurred at 3 and 7 days following ICbH.

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ABSTRACT

The present study aimed to investigate the behavior and neuronal morphological changes in the perihaemorrhagic tissue of the mouse intracerebellar haemorrhage experimental model. Adult male Swiss albino mice were stereotactically infused with collagenase type VII (0.4 U/μl of saline) unilaterally in to the cerebellum, following anaesthesia. Motor deficits were assessed using open field and composite score for evaluating the mouse model of cerebellar ataxia at 1, 3, 7, 14 and 21 days after collagenase infusion. The animals were sacrificed at the same time interval for evaluation of perihaematomal neuronal degeneration using haematoxylin and eosin staining and Annexin V-FITC/Propidium iodide assay. At the end of the study, it was found that infusion of 0.4 U collagenase produces significant locomotor and ataxic deficit in the mice especially within the first week post surgery, and that this gradually improved within three weeks. Neuronal degeneration evident by cytoplasmic shrinkage and nuclear pyknosis was observed at the perihaematomal area after one day; especially at 3 and 7 days post haemorrhage. By 21 days, both the haematoma and degenerating neurons in the perihaematomal area were phagocytosed and the remaining neuronal cells around the scar tissue appeared normal. Moreover, Annexin-V/propidium iodide–positive cells were observed at the perihaematomal area at 3 and 7 days implying that the neurons likely die via apoptosis. It was concluded that a population of potentially salvageable neurons exist in the perihaematomal area after cerebellar haemorrhage throughout a wide time window that could be amenable to treatment.

Abbreviations: CH, Cerebellar haemorrhage; ICH, Intracerebral haemorrhage; IVH, Intraventricular haemorrhage; IVF, Intraventricular fibrinolysis; IVD, Intraventricular drainage; CE, Clot evacuation; NMDA, N-Metthyl-D-Aspartic acid; IL-1β, Interleukin 1 beta; TNF-α, Tumour necrosis factor alpha; CNS, Central nervous system; CSF, Cerebrospinal fluid; FITC, Fluorescein isothiocyanate; TUNEL, Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling; OFT, Open field test; OF, Open field; MAO, Medial accessory olive; DAO, Dorsal accessory olive; MAOr, Rostral medial accessory olive; DAOr, Rostral dorsal accessory olive; MedM, Rostromedial nucleus; MedCm, Caudomedial nucleus; MedDL, Dorsolateral nucleus; 1Cb, 1st Cerebellar lobule 1Cb; 2Cb, 2nd Cerebellar lobule; 3Cb, 3rd Cerebellar lobule; 4&5Cb, 4&5th Cerebellar lobules 4&5Cb; Med, medial (fastigial) cerebellar nucleus; IntA, interposed cerebellar nucleus anterior part; Lat, lateral (dentate) cerebellar nucleus; VeCb, vestibulocerebellar nucleus.

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1. Introduction

Cerebellar haemorrhages account for approximately 10% of all intracerebral haemorrhages (ICH) in most populations, and nearly 50% of cerebellar strokes [12]. Most common in the elderly population, the average age of onset reported in most series is greater than sixty years [4,51]. Few cases which are often due to angiomatous malformations however exist among teenagers [22]. Improved clinical imaging and early surgical intervention have contributed largely to the decreased mortality rate from cerebellar haemorrhages in recent years [33], compared to that of decades ago [31]. However, the long term functional outcome in the majority of the surviving patients remains unfavourable, regardless of whether they are treated surgically or conservatively [4,51]. Nevertheless, the pathophysiological mechanisms contributing to neuronal loss after cerebellar haemorrhage are not understood yet.

The use of animal models has been very influential to the understanding of pathogenesis of injury after haemorrhage in the brain [5,24]. Stereotactic infusion of the bacterial collagenase enzyme constitutes an important method of inducing haemorrhage in experimental animals. When injected into the brain parenchyma, collagenase dissolves into the extracellular matrix sorrounding the capillaries and opens the blood-brain barrier (BBB), thus inducing intracerebral bleeding [39]. The advantage of the collagenase haemorrhage model is that it generates a spontaneous and gradually expanding haematoma which mimics the human condition, with histological changes that are also consistent with the changes noted in human brain tissue after intracerebral haemorrhage [6,27]. Taking advantage of the extensive studies done with bacterial collagenase in basal ganglia lesions, Lekic and colleagues developed and characterized a collagenase-induced cerebellar haemorrhage model by infusion of 0.6 U collagenase into the paramedian white mater of the rat's cerebellum [23]. Adopting from the work of Lekic, we reproduced a collagenase-induced cerebellar haemorrhage in mice and subsequently studied the perihaematomal neuronal degeneration over a 21 day period.

Mice are increasingly adopted as animals of choice in intracerebral haemorrhage research [8,50]. It is believed that genetically engineered mice are valuable tools for determining the mechanism of injury following intracerebral haemorrhage [26]. Through the use of knock-out or transgenic mice, numerous studies have recently identified the detrimental effect of various factors such as superoxide, heme oxygenase 1, Toll-like receptor 4, signal transduction molecules such as Caveolin-1, and heme in supratentorial intracerebral haemorrhage [3,6,41,46]. Moreover, using a similar approach, the neuroprotective potential of other agents including heme-oxygenase 2, aquaporin-4 expression, haptoglobin and elements of the complement system in intracerebral haemorrhage have been identified [8,32]. Therefore, characterizing a model of intracerebellar haemorrhage in mice could be a gateway to the understanding of injury mechanisms and potential therapeutic targets after cerebellar haemorrhage.

2. Materials and methods

2.1. Animals

Adult male Swiss albino mice (25–35 g) were obtained from the Animal Research and service centre (ARASC), University Sains Malaysia, Health Campus, Kuban Kerian, Kelantan. All mice were maintained in ARASC. They were kept in polypropylene cages (32 cm × 24 cm × 16 cm), exposed to 12 h light dark cycles and supplied with a pellet diet and water ad libitum. Paddy husk was used as bedding material and was changed in two day intervals.

2.2. Experimental design

Animals were divided into seven groups: Group I (n = 6) served as a control without induction of cerebellar haemorrhage, Group II (n = 6) served as a sham and were injected with sterile saline only, while Group III, 1 day (n = 6), Group IV, 3 days (n = 6), Group V, 7 days (n = 6), Group VI, 14 days (n = 6) and Group VII, 21 days (n = 6) were all induced with cerebellar haemorrhage using the bacterial collagenase type VII enzyme. Their motor behaviours were assessed at 1, 3, 7, 14 and 21 days post haemorrhage and they were sacrificed at the same time intervals for morphological studies.

2.3. Operative procedure

36 age and weight matched adult male (25–35 g) Swiss albino mice were used for the study. All procedures were in compliance with the Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of University Sains Malaysia, Health Campus (Animal ethics approval/2014/(92)/(548)). Aseptic technique was used for all surgeries. Mice were anesthetized with isoflurane (3% induction, 2% maintenance, 1 l/min O2) and secured prone onto a stereotaxic frame (C.H. STOELTING Co. Stellar Stereotaxic Instrument, Model 51400, Lorton, VA) before making an incision over the scalp. The following stereotactic coordinates were then measured from the bregma, to locate the deep cerebellar (paramedian) white matter tract: −5.8 mm (caudal), 2.0 mm (lateral), and 3.0 mm (deep). A borehole (1 mm) was drilled, and a 30-gauge needle was inserted. Collagenase type VII (0.4 U/μL, Sigma, St Louis, MO) was infused via a microinfusion pump (rate = 0.2 μl/min, Harvard Apparatus, Holliston, MA). The syringe remained in place for 10 min to prevent back-leakage before being withdrawn. The borehole was then sealed with bone wax (World Precision Instruments Inc.), and the incision was closed using Nexaband liquid topical tissue adhesive (Abbott Labs), allowing time for the animals to recover. Control surgeries consisted of sterile saline injection alone. The animals were given free access to food and water upon recovery from anaesthesia.

2.4. Behavioural assessment

Locomotion activity and ataxia were assessed using open field and composite phenotype scoring system for evaluating mouse model of cerebellar ataxia respectively.

2.5. Locomotor activity

Locomotor activity in both the control, sham-operated and the collagenase injected mice was assessed at 1, 3, 7, 14 and 21 days after the surgery by means of an open-field apparatus as previously described [9]. The apparatus consisted of a Perspex cage (height: 40 cm, length: 90 cm; width: 90 cm), with the bottom divided into 25 small squares (16 cm × 16 cm). A video camera was placed 250 cm above the open field to record trials (Arc Soft Total media 3.5). For testing, each mouse was placed in the centre of the open field and locomotor activity was digitally recorded for 5 min. The open field floor was wiped with 30% ethanol between trials and allowed to dry before the next trial, as is standard protocol in open-field testing. The number of lines crossed and numbers of rearing were manually counted offline for each mouse.

2.6. Ataxia

Ataxia was assessed using the composite phenotype scoring system for evaluating mouse models of cerebellar ataxia. The different measures in this scale include hind limb clasping, ledge test and