ASSESSMENT OF NEURONAL AND GLIAL INJURY IN A RODENT MODEL OF FOCAL ISCHEMIA

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AIM

We adapt a mouse stroke model to study the pathological changes in the evolution of an infarct in both gray and white matter regions.

INTRODUCTION

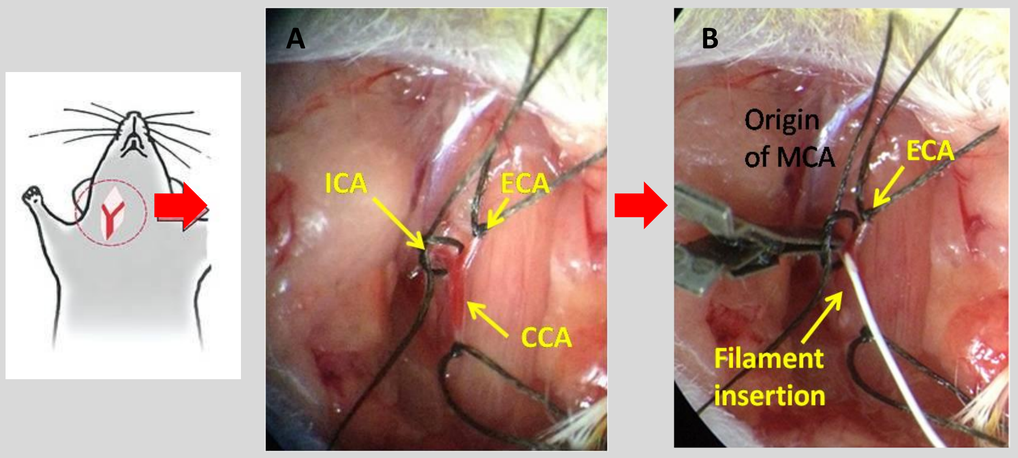
Human stroke affects equivalent volumes of gray and white matter. Traditionally, focus on assessment of ischemic injury in experimental models was dominated by histological assessment of neuronal viability and cell counts. However evidence has demonstrated the functional, behavioral and long-term impact of white matter lesions in stroke and therefore, a much needed effort to address the integrity of both gray and white matter lesions is important in devising strategies to protect the brain as a whole.

Focal cerebral ischemia models simulating human stroke are indispensable in investigating cerebral gray and white matter injury.

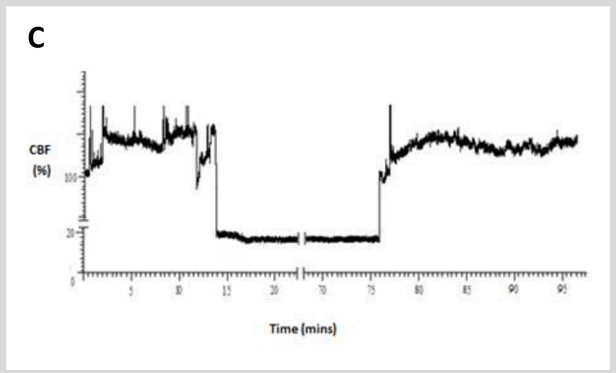
The intraluminal filament mouse middle cerebral artery occlusion (MCAO) model mimics one of the commonest causes of stroke in humans[1] and is one of the most frequently used models in experimental stroke research.

METHOD AND RESULTS

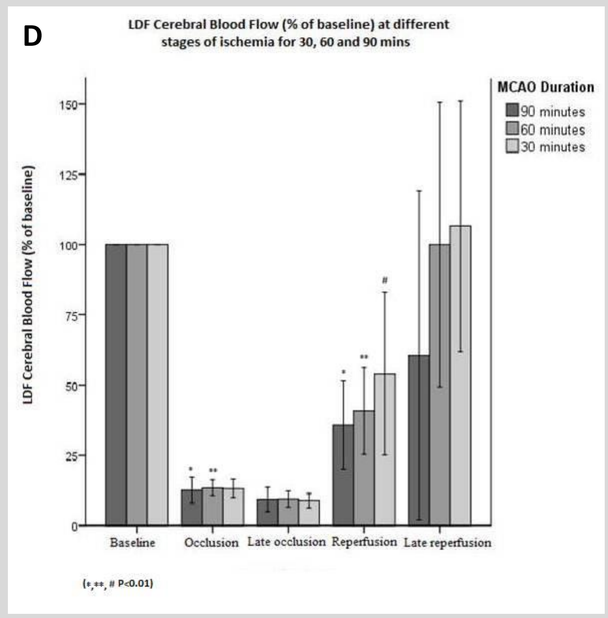
INTRALUMINAL FILAMENT MCAO MODEL



(A, B) Following isoflurane anaesthesia induction, the common carotid artery (CCA) and external carotid artery (ECA) were isolated and ligated. A silicon-coated intraluminal filament was introduced into the CCA close to the CCA-ECA bifurcation and advanced upwards into the internal carotid artery (ICA) to occlude the origin of the middle cerebral artery (MCA). Following a set occlusion duration time, the filament was withdrawn, allowing reperfusion.



(C) Successful occlusion was confirmed by means of Laser Doppler Flowmetry (LDF), which measures local circulatory blood perfusion thus allowing acquisition of hemodynamic changes in superficial brain structures. An LDF probe was affixed to the skull, 5mm caudal and 2mm lateral to bregma in order to measure relative cerebral blood flow (rCBF) around the core area of the MCA territory. Measurement commenced prio to occlusion, after which a steady baseline was achieved (a). Upon successful filament insertion, rCBF dropped to approximately 85% of baseline in most cases (b), and remained as such throughout the duration of occlusion. Filament retraction was followed by restoration of blood flow as confirmed through LDF (c).



(D) LDF measurements for rCBF at baseline, throughout ischemia and reperfusion for 30, 60 and 90 minutes occlusion. rCBF dropped comparably from baseline upon filament insertion ($p<0.001$; ANOVA) and remained low throughout the duration of ischemia. Blood flow was restored upon filament withdrawal to different levels depending on the duration of ischemia.

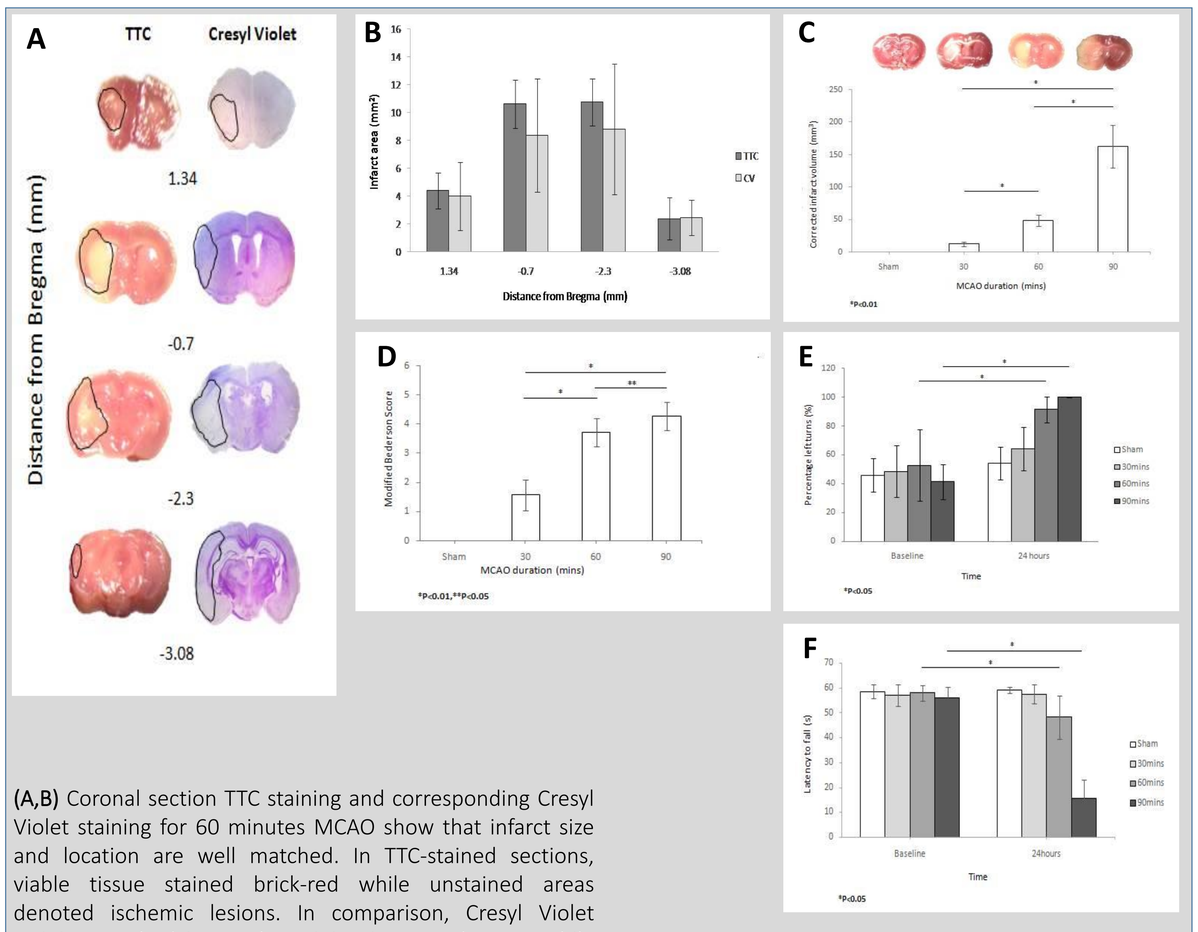
HISTOPATHOLOGY

|| Determination of ischemic lesion volumes was performed 24 hours after reperfusion. The brain was quickly dissected and coronal sections were taken at 1.34mm rostral, -0.7mm, -2.3mm and -3.08mm caudal from the bregma. Sections were immersed in 2% 2,3,5-triphenyltetrazolium (TTC) stain, which stained viable tissue red while infarcted tissue remained unstained (white).

|| Assessment of neuronal damage was done by the Nissl stain Cresyl Violet on previously fixed tissue. Viable tissue stained dark purple/blue while the lesion area, with less intact cells than normal, stained light purple/blue.

|| Luxol Fast blue staining (LFB) was used for assessment of myelin integrity. In viable tissue, myelinated fiber tracts showed morphologically intact fibers with intense staining. Loss of LFB staining and compromised myelin structure was seen in damaged myelinated regions.

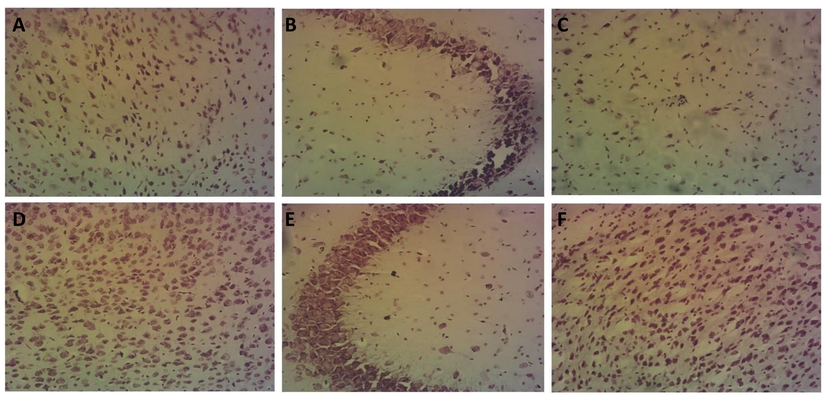
|| Evaluation of neurological function was performed by a series of behavioural tests conducted pre- and post-surgery to assess sensory and motor function. These included the Modified Bederson Score, Corner test and Wire Hanging test.

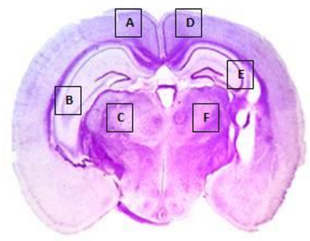


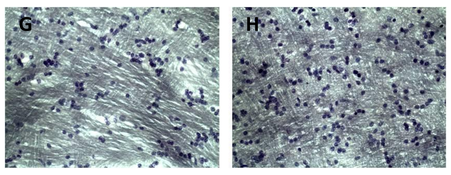
(A,B) Coronal section TTC staining and corresponding Cresyl Violet staining for 60 minutes MCAO show that infarct size and location are well matched. In TTC-stained sections, viable tissue stained brick-red while unstained areas denoted ischemic lesions. In comparison, Cresyl Violet staining resulted in purple staining in normal areas, while neuronal loss and disruption translated to decreased staining intensity.

(C-F) High grade neurological deficits observed from behavioral tests were predictive of consistent histopathological infarction. Infarct volumes for different occlusion durations: sham, 30, 60, 90 minutes (C) corresponded to severity of deficits as seen from Modified Bederson score (D), Corner test (E) and Wire hanging test (F).

(A-F) Cresyl Violet stained coronal section for assessment of neuronal damage. Histological appearance of infarct regions denotes neuronal loss and overall pale staining of the neuropil, especially in the motor cortex (A) and striatum (C), cytoplasm vacuolation (B), nuclear scalloping and cell shrinkage (A-C), compared to contralateral regions inclusive of the hippocampus (D-F).

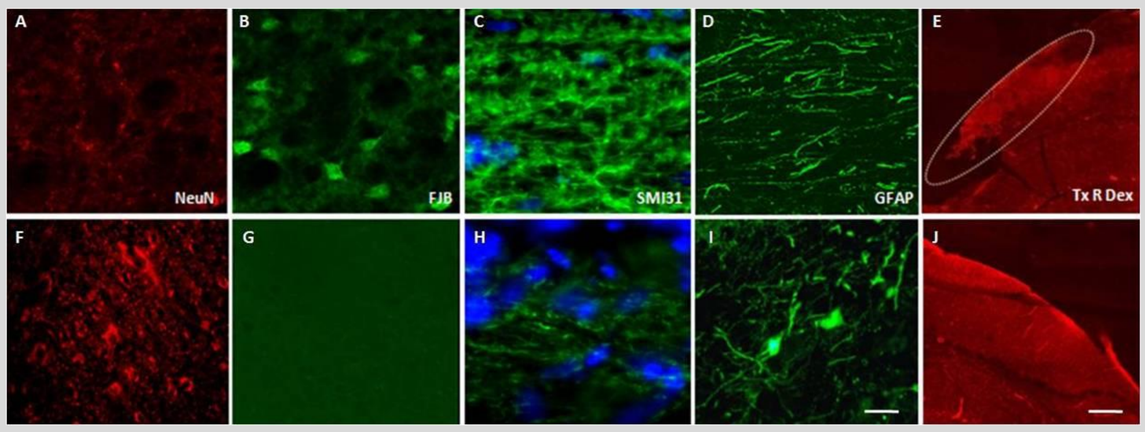






(G) Luxol Fast Blue staining exhibited disordered fiber bundles of white matter and vacuole-like structures surrounding axons in the ipsilateral corpus callosum. (H) Intact myelin seen in the contralateral area.

MARKERS OF GRAY AND WHITE MATTER INJURY



(A, F) NeuN immunoreactivity was used as a specific marker for neurons. NeuN immunostaining of motor cortex after 60min MCAO showed a decrease in intensity with only a few cells being NeuN-positive in the lesion area (A) compared to the contralateral hemisphere (F), indicating CNS injury. (B,G) FluoroJade B was used as a marker of degenerating neurons. Injured neurons are recognized by the proficient uptake of the fluorescencent dye (B) compared to the contralateral side (G). (C, H) Double staining with Hoechst, a nuclear stain and SMI31, a marker of axonal damage. SMI31 immunoreactivity showed loss of staining and the formation of axonal heads and retraction bulbs together with pyknotic nuclei in the ipsilateral corpus callosum in comparison to the intact and linear axonal profiles and preserved nuclei in the contralateral white matter (D, I) GFAP was used as an astrocytic marker. (D) shows reduced GFAP immunoreactivity in ipsilateral corpus callosum accompanied by fragmented processes as compared to the intact astrocytes with the non-ischemic contralateral region. (E, J) Loss of dye-filled blood vessels and tissue disintegration was visualised in the ipsilateral MCA territory after IV injection of Texas Red dextran. Dye extravasation was also visualized seen in the injured motor cortex (E) as a result of increased permeability in comparison to the intact contralateral hemisphere. (Scale on I=20um for A-I, scale on J=1mm).

CONCLUSION

The intraluminal suture MCAO model provides reproducible MCA territory infarction and and is a powerful tool for assessment of both gray and white matter damage and studies of brain repair.

References:
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2) Hattori K, Lee H, Hurn PD, Crain BJ, Traystman RJ, DeVries AC. Cognitive deficits after focal cerebral ischemia in mice. Stroke. 2000; 31:1939–1944.
3) Clark W, Lessov N, Dixon M, Eckenstein F. Monofilament intraluminal middle cerebral artery occlusion in the mouse. Neurol. Res. 1997;19:641-648.