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Acute ischaemic lesion evolution: potential sex differences in a rodent model of stroke

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Summary

Ischaemic stroke occurs when blood flow through a major cerebral blood vessel is blocked or severely reduced. Severe reductions in cerebral blood flow trigger a cascade of events culminating in irreversibly damaged brain tissue. Following stroke onset, brain-injured tissue can be assessed using magnetic resonance imaging (MRI).

Applying a fixed threshold to accurately predict stroke-induced brain damage in males and females is important for therapeutic decision making, especially since men and women are known to respond differently to stroke.

Here, we show using a rodent stroke model, that at 24hrs after stroke, females experience less brain tissue injury compared to males despite being exposed to the same insult. The defined threshold values, below which tissue is destined to die, did not differ greatly between the sexes. However, the rate of lesion growth was significantly higher in males versus females over the first hour after stroke.

The data show that experimental stroke induces less brain damage in female compared to male rats and lesion growth was significantly slower in females in the first hour after stroke.

Introduction

- Diffusion-weighted imaging (DWI) is widely used to assess brain injury in acute stroke patients.
- An ischaemic insult unleashes a series of pathophysiological mechanisms including disruption to energy metabolism leading to the movement of water from the extracellular to intracellular space causing cellular swelling. DWI assesses ischaemic injury by detecting changes in the apparent diffusion coefficient (ADC) of brain water. ADC maps provide a quantitative estimate of the restricted diffusion of water molecules.
- Low ADC values in ischaemic tissue coincide with the onset of cytotoxic oedema[1] and reflect the development of ischaemic damage following stroke.
- Establishing ADC thresholds to accurately predict tissue at risk of infarction is of critical importance in therapeutic decision making. Applying fixed thresholds that are too low or too high may result in under- or over-estimation of infarcted tissue.
- Gender differences are known to exist in stroke. For example, premenopausal women experience a lower incidence of stroke compared to men[2]. Similarly, in experimental stroke studies, intact female rodents exhibit smaller infarcts compared to age-matched males[3]. Defining accurate ADC thresholds for both males and females is important since very little is known about sex differences in the evolution of ischaemic injury over the first hours after stroke

Methods

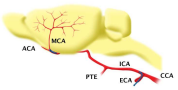


Figure A

Animal preparation

- Male Sprague-Dawley rats (300-350g)
- Anaesthesia: isofluorane: 5% induction, 2.5-3% maintenance
- Surgical tracheostomy and mechanical ventilation
- Femoral arteries (2) cannulated for MABP and arterial blood gas analysis
- Focal cerebral ischaemia induced by middle cerebral artery occlusion (MCAO) using the intraluminal filament model

MRI scanning protocol

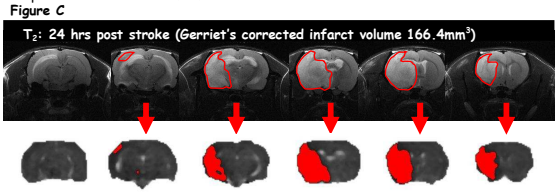
- Rats were immediately transferred to a Bruker Biospec (7T/30cm) MRI scanner equipped with a 72mm birdcage resonator and a 2cm surface coil placed on the head.
- To track evolution of the ischaemic lesion, a spin echo based Echo Planar DWI scan (effective echo time (TE)=22.5ms; repetition time (TR)=4000.3ms; 4 averages; matrix=96x96; field of view (FOV)=25x25 mm; 3 directions_x, y, z; B values=0 and 1000 s/mm2; 6 contiguous slices; 1.5-mm thickness) was carried out every hour for 4 hours (see Figure B).
- 24 hours later, rats underwent a RARE T_2 scan (TE=47.2ms, TR=5000 ms, 4 averages, matrix=256x256, FOV=25x25 mm, 16 contiguous slices, 0.75-mm thickness) to determine final infarct size.

Figure B

Analysis

- Using RARE T_2 images, infarct volumes (corrected for hemispheric swelling using Gerriet's correction factor[4]) were calculated at 24 hrs post-MCAO .
- A range of ADC threshold values were applied to ADC maps 4 hours post-MCAO until the ADC-defined lesion volume at 4 hrs numerically matched RARE T_2-defined infarct volume at 24 hrs (see Figure C).
- Threshold values were then applied to early time points to define lesion volume at 0.5, 1, 2 & 3 hrs post-MCAO. ADC pixels below the fixed threshold were considered to represent tissue at risk of infarction.

Figure C



ADC Map: 0.54 x10^{-3} mm^2/sec threshold which matches T_2 infarct volume (168 mm^3)

Results

1. Females exhibit smaller infarct volumes at 24 hrs

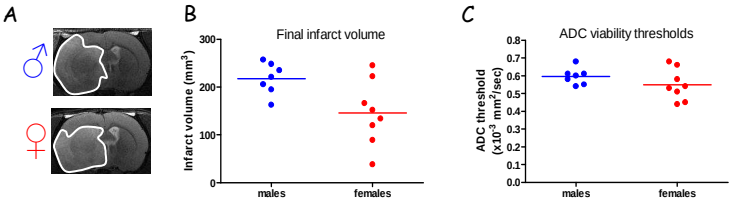


Figure 1: (A) Representative RARE T_2 images delineating final infarct size (outlined in white) at 24 hrs in a male and female rat. (B) Scatterplot of RARE T_2-defined infarct volume at 24 hrs and (C) calculated ADC thresholds yielding similar lesion volumes at 4 and 24hrs (males, n=7, ADC 0.6±0.05x10^{-3}mm^2/s; females, n=8, 0.55±0.09x10^{-3}mm^2/sec).

2. Spatiotemporal progression of ADC lesion

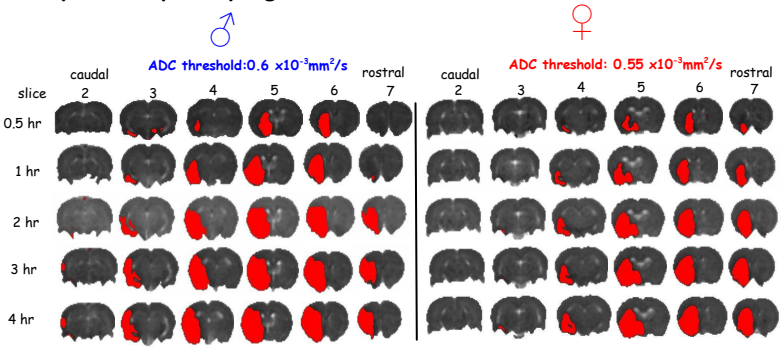


Figure 2: Representative ADC maps with (group average) thresholds applied, tracking spatiotemporal evolution of the ADC lesion in a male (left) and female (right) rat. Six coronal slices (caudal-rostral, 2-7) throughout MCA territory are shown at 0.5, 1, 2, 3, and 4hrs after onset of ischaemia

3. Differences in ischaemic lesion growth between the sexes

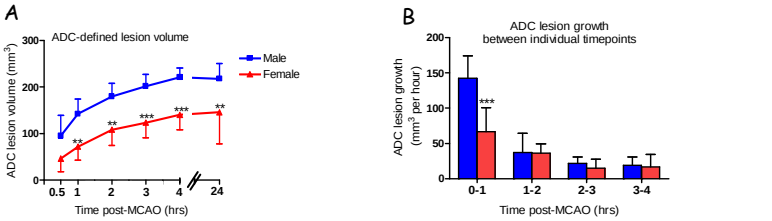


Figure 3: Evolution of the ischaemic lesion, data are presented as mean±SD. (A)Temporal evolution of the ADC-derived lesion volume, (B)ADC lesion growth between 0-1, 1-2, 2-3 and 3-4 hrs post-MCAO in males and females (**P<0.01; ***P<0.001 vs males, two-way ANOVA with Bonferroni post test) .

Main findings

- In-house ADC thresholds defined by volumetric matching of ADC lesion volume at 4 hrs to T_2 infarct volume at 24hrs post-MCAO revealed similar ADC values (below which tissue is destined to die) between the sexes and comparable with other published viability thresholds[5].
- ADC derived lesion volumes were significantly smaller in females from as early as 0.5 hrs post-stroke and remained so throughout the entire 24 hour time course.
- Lesion growth was significantly slower in females compared to males over the first hour post-MCAO.

References

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2. Barrett-Connor & Bush, 1991, *JAMA*, 265:1861-1867.
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Acknowledgements

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