Ischaemic Preconditioning in the Rat Brain: A Longitudinal Magnetic Resonance Imaging (MRI) Study.

Paul G Mullins[1], David G Reid[2], Paul Hockings[2], Sarah J Hadingham[3], Colin A Campbell[3], Jonathan Chalk[4], David Doddrell[4]
[1]University of New Mexico, CMRRC, 1201 Yale Blvd NE, Albuquerque, NM USA; [2]SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, England, UK; [3]SmithKline Beecham Pharmaceuticals, Harlow, Essex UK; [4]University of Queensland, Centre for Magnetic Resonance, Brisbane, QLD Australia;

Introduction

Ischaemic preconditioning is the induction, by a short sub-lethal ischaemic attack, of tolerance to further episodes of ischaemia in the affected tissue. Chen *et al* (1) have shown that 30 minutes of ischaemia provides neuroprotection at 72 hours after a second ischaemic insult. However, the effects of ischaemic preconditioning on temporal lesion volume evolution are largely unknown, but could be readily studied non-invasively by MRI. We have measured the initial lesion produced by a short ischaemic insult, and then followed the evolution of injury after a second longer and more severe period of ischaemia. As such this study reports the first use of T2-weighted (T2W) MRI to follow lesion maturation in rats subjected to ischaemic preconditioning , followed 3 days later by a second, longer, period of ischaemia.

Methods

Using the intraluminal filament method (2) MCAO was performed in Sprague-Dawley male rats (300-350g; n=8 per group). Ischaemic preconditioning was induced by 30 minutes MCAO while a sham group underwent identical surgery without filament insertion. A second insult of 100 minutes MCAO was induced in all rats 3 days after induction of ischaemic or sham-preconditioning, by reintroduction of the filament in a manner similar to the preconditioning episode.

Animals were imaged using a three dimensional (3D) fast spin echo technique. The 3D T2W multi-echo image was acquired using a rapid acquisition with relaxation enhancement (RARE) sequence (3) (32 echoes, no averages) with a (3 x 3 x 3)cm^3 FOV and 128 x 128 x 64 resolution zero filled to $(128)^3$. An inter-echo time of 6.5 ms was used, which with the RARE factor of 32 gave an effective TE of 104 ms. All images were obtained with respiratory gating (4) leading to an effective repetition time (TR) of approximately 1 s. Animals were anaesthetised and maintained during imaging with isoflurane at 5% for induction, and 1.8% for maintenance in carrier gas (40% N2 / 60% O2).

Imaging was performed at 24 hours following ischaemic or sham preconditioning and again at 24 and 72 hours after the secondary insult. Lesion volume was determined by a semi-automated method using Bruker ParaVision software.

Following the final imaging session, rats were sacrificed and transcardially perfused with neutral buffered formalin (NBF). After immersion of the head in NBF for 24h brains were removed for histological processing. All data are presented as mean ± standard error. Student's two-tailed t-test, assuming unequal variance, was used for statistical comparisons between groups, and ANOVA analysis of variance was used for within group analysis, with $p<0.05$ taken to indicate significant differences.

Results

At 24 hours after the preconditioning stimulus, preconditioned animals typically showed hyperintense sub-cortical lesions, with no cortical damage, while in sham preconditioned animals the entire ipsilateral hemisphere was unaffected. At twenty-four hours and 72h following the secondary insult preconditioned animals showed significantly smaller lesions, which were confined to the striatum, than controls (figure 1).

In addition, during lesion maturation from 24h to 72h post-secondary MCAO, preconditioned rats displayed an average reduction in lesion size as measured by MRI whereas sham-preconditioned rats displayed increases in lesion size. MRI measures at 72 hours after the second insult correlated strongly with histological measures at the same time point ($r^2 = 0.928$).

Discussion

We confirm that brief (30 minute) focal cerebral ischaemia produces considerable tolerance to subsequent insult several days later (1). Uniquely, however, the non-invasive nature of MRI enabled the study of the effects of the preconditioning episode itself, as well as lesion maturation, in individual subjects. Thus MRI enabled the observation that the preconditioning event induced small sub-cortical lesions, and that post-secondary lesion volume diminished between 24 hours and 72 hours in ischaemic preconditioned rats compared to sham-preconditioned rats in which progressive increases in lesion volume were noted. This implies that lesion measurement at 24 hours by MRI is not necessarily definitive of final infarct size and highlights the advantage of serial measurements of lesion growth. Identification of the mechanisms involved in preconditioning could lead to effective pharmacological neuroprotective interventions, and may also elucidate neuroreparative processes.

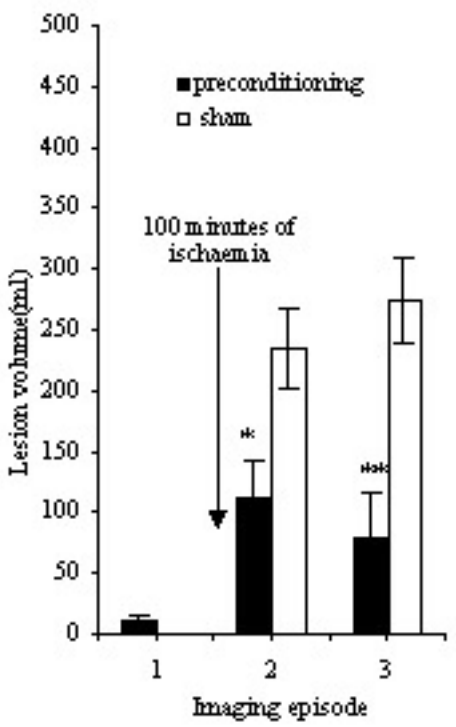

Figure 1

Mean lesion volumes after 30 minutes of MCAO (preconditioning) or sham MCAO followed by a further 100 minutes of MCAO three days latter (bars show standard errors).

References

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