NMR imaging of the neuroprotective effects of estrogen

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Introduction

Stroke is the third leading cause of death and the most common cause of adult disability in the United States with 500,000 new cases per year[1]. Thrombosis of the middle cerebral artery (MCA) is a common cause of stroke, leading to a cascade of neuronal and microvascular changes, ultimately resulting in infarction. There is ample evidence that ischemic cell injury occurs during the occlusive phase, but reperfusion injury is also an important factor, especially when reperfusion follows prolonged ischemia. Since new thrombolytic agents can now restore arterial patency, the development of techniques to rapidly detect an ensuing stroke becomes paramount. In addition, efforts to blunt reperfusion-associated injury and extend the therapeutic window for thrombolysis have resulted in a search for new neuroprotective agents.

In this study, we combine these two drives with the application of diffusion-weighted NMR imaging[2] to characterize in a dynamic and non-invasive fashion, the potential neuroprotective effects of estrogens in a transient MCA occlusion model of focal ischemic stroke.

Methods

Seven female Sprague-Dawley rats (200-225 grams) were used for these experiments. Two weeks prior to MCA occlusion, all rats were ovariectomized to eliminate endogenous estrogens. Rats in the experimental group (n=3) were administrated a single dose of 17 β-estradiol (E_2) (100 μg /kg) two hours before MCA occlusion, while those in the control group (n=4) did not receive estrogen replacement.

MCA occlusion was achieved as previously reported[3,4]. Briefly, animals were anesthetized, and a midline cervical incision exposing the left common, external and internal carotid arteries was performed. A 3-0 monofilament suture was introduced into the internal carotid artery and advanced to the bifurcation of the anterior cerebral artery and MCA until resistance detected, thus indicating MCA occlusion. The suture was kept in place for 60 min and then withdrawn to allow MCA reperfusion.

NMR images were collected on a 4.7 T 33-cm magnet with a Bruker console, using an actively shielded gradient set. The animals were supported on a cradle and their heads placed in a home-built birdcage coil with a 5-cm outer diameter (operating in quadrature transmit/receive mode). Following the acquisition of scout images, 6 coronal plane images were prescribed beginning 3 mm behind the olfactory bulb. Diffusion-weighted (DW) images were acquired using a standard pulsed gradient spin echo technique with a TR of 1.75 s, TE of 33 ms, a 5 cm field of view with a 128 x 128 matrix (0.39 x 0.39 mm in-plane resolution), a slice thickness of 1.5 mm and 2 averages. Each set of 6 images was acquired in 7.5 min. The gradient pulses were each applied for 9 ms and were separated by 13 ms around the 180° refocusing pulse. The gradient amplitude used was 152 mT/m resulting in a b-value of 1400 s/mm^2. Images were captured sequentially for each animal 30 min into MCA occlusion (the occlusion phase), and 2, 4, 6 hours after withdrawal of the monofilament (the reperfusion phase).

Results

DW imaging detected early changes in lesion sizes at 30 min into MCA occlusion. The total lesion size in the occlusion phase was similar in both groups (33.7% and 33.5% of the whole hemisphere in the control and E_2 treated groups, respectively), but was larger in cortical regions in the control group (26.5% vs. 17.1% in E_2 treated). During the reperfusion phase, the lesion size remained constant in the control group but decreased in the E_2 treated group by 50-60% ($p<0.05$). This lesion size reduction was maintained at all time points after reperfusion (2, 4, and 6 hours). This size reduction was primarily located in cortical regions. Representative images are shown in figure 1.

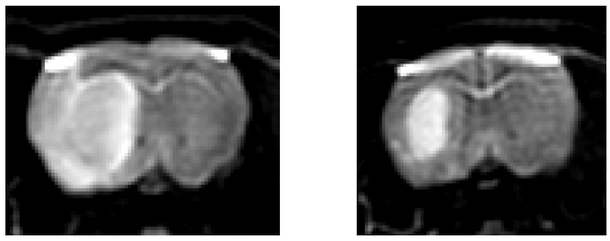

Fig. 1 Representative DW images 4 hours after reperfusion. The left panel indicates extensive lesion induced signal enhancement after reperfusion in a control animal. With E_2 treatment, right panel, lesion size was markedly reduced and confined to subcortical areas.

Discussion

In this study, diffusion-weighted NMR imaging was applied to study the role of estrogens in modulating the temporal and spatial evolution of damage in a focal ischemic stroke model. It demonstrates that estrogens selectively protect cortical tissue from ischemic damage and that this protection is primarily exerted during the reperfusion phase of damage.

Reperfusion of ischemic tissue can result in accumulation of oxygen-derived free radicals. This oxidative stress may compromise plasma membrane integrity, which in turn could increase Ca^{2+} influx into the cell, resulting in further neuronal death. Estrogens can attenuate free radical-induced peroxidative damage, modulate Ca^{2+} homeostasis, increase brain glucose utilization and glucose receptor expression which together may account for some of the neuroprotective effects of estrogens[3]. This neuroprotection is particularly important in the stunned ischemic penumbra[4].

Studies indicate that thrombolytic therapy may be effective against ischemic damage provided that treatment is initiated within 3 hours[5]. This study suggests that estrogens may be useful in widening this therapeutic window by protecting against thrombolysis-induced reperfusion injury and potentially have direct clinical applications.

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