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Ischemic preconditioning induces XRCC1, DNA polymerase-β, and DNA ligase III and correlates with enhanced base excision repair

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ABSTRACT

Neuronal protection induced by ischemic preconditioning has an important role in the reduction of stroke volume and attenuation of neuronal cell death. Ischemic injury is associated with increased oxidative DNA damage, and failure to efficiently repair these oxidatively damaged lesions results in the accumulation of mutations and neuronal cell death. Although the effects of ischemic tolerance can have profound implications, the precise mechanisms mediating this phenomenon remain unclear. The base excision repair (BER) pathway has a major role in the repair of oxidative DNA base damage after ischemic injury. Using a rat model of ischemic preconditioning, we now report that the neuronal protection observed after induction of ischemic tolerance is associated with increased BER. *In situ* detection of single-strand breaks and apurinic/apyrimidinic sites reduced to baseline levels after reperfusion following ischemic preconditioning. By contrast, no change was seen in the quantity of *in situ* lesions after reperfusion in non-ischemic preconditioned brain. Induction of the BER proteins XRCC1, DNA polymerase-β, and DNA ligase III was seen after reperfusion in ischemically conditioned brain. Moreover, an increase in binding between XRCC1 and DNA polymerase-β was seen under these conditions, as might be expected during formation of functional BER complexes. Using *in vitro* BER oligonucleotides, we directly demonstrated an increase in total BER capacity of nuclear extracts prepared from ischemic-conditioned brain after reperfusion compared with sham-operated brain. These findings provide direct evidence that increased BER is associated with the neuroprotection induced after ischemic preconditioning, and provides important new mechanistic insight into the important biologic pathways that protect neurons against irreversible ischemic injury.

1. Introduction

Ischemic tolerance is a phenomenon by which a sub-lethal (or mild) ischemic episode protects the brain from subsequent severe ischemia. This phenomenon is recognized in global and focal cerebral ischemia, as well as in neuronal cultures [1–8]. The neuronal protection afforded by preconditioning can reduce the stroke volume and prevent neuron loss *in vivo* after focal and global ischemia, and reduce neuronal death in *in vitro* ischemia models [9–12]. Several mechanisms have been proposed to mediate ischemic brain injury, although their precise roles in ischemic tolerance remain unclear [8,13–18].

Oxidative DNA damage is markedly induced in the brain within minutes of cerebral ischemia, and cells are at risk of

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developing DNA lesions and triggering cell death if the rate of oxidative damage is greater than its repair [12,19–26]. Upregulation of DNA repair capacity may underlie an important mechanism of endogenous neuroprotection, and failure in such response may, in part, lead to cell death [20,25,27]. Base excision repair (BER) is a process that recognizes and repairs damaged modified bases [28], oxidative base damage, multiple forms of alkylation damage, apurinic/apyrimidinic (AP) sites formed by the spontaneous loss of bases, and uracil residues in some of the DNA lesions that are substrates for BER [29,30]. Recent reports have described an increased mitochondrial BER capacity in rat brain after brief ischemia compared with prolonged ischemia [31,32]. However, it remains unclear whether a similar increase in nuclear BER occurs due to ischemic preconditioning. X-ray repair cross-complementing group 1 protein (XRCC1) has an important role in the DNA BER pathway [33,34]. XRCC1-mediated DNA repair is dependent on formation of DNA repair complexes consisting of XRCC1, DNA polymerase-β, poly-ADP-ribose polymerase, and DNA ligase III [35–38]. BER is a major DNA repair mechanism in the brain after ischemic injury [39,40]. Reduced levels of XRCC1 protein may contribute to neuronal damage after focal ischemia in mice [33,41]. However, the roles of XRCC1 and other DNA BER enzymes in ischemic tolerance *in vivo* remain largely unknown.

Moreover, recent studies also show that ischemic preconditioning increased levels of the DNA repair protein Ku and reduced the oxidative DNA damage following subsequent lethal global ischemia [42]. These results indicate that activation of the endogenous DNA repair mechanism may contribute to the induction of ischemic tolerance.

To investigate whether ischemic tolerance could be correlated with the induction of the BER enzymes XRCC1, DNA polymerase-β, and DNA ligase III, we analyzed the expression of these enzymes and DNA damage in the cortex using a rat model of ischemic preconditioning. In this model, tolerance is induced by subjecting animals to 30 min of middle cerebral artery occlusion (MCAO) 72 h before a harmful 100-min MCAO. Assessment of infarct is performed 24 h after the harmful ischemic insult. The model and different infarct area are shown in Fig. 1. We also sought to clarify the relationship between the expression of BER enzymes and the repair of damaged DNA. Our results indicate that induction of DNA repair enzymes and increased BER activity after ischemic preconditioning might have an important role in focal cerebral ischemic tolerance.

2. Results

2.1. *Ischemic preconditioning reduces neuronal damage after harmful ischemia*

In this study, we investigated the role of XRCC1, DNA polymerase-β and DNA ligase III in mediating the reduction of DNA damage after preconditioning in a focal model of ischemic tolerance. The ischemic preconditioning model is shown in Fig. 1A, and several previously published studies have used this *in vivo* rat tolerance ischemic model [39,43,44]. Ischemic infarct size in rats after MCAO was determined using the vital dye TTC. Cortical infarction after a 100-min MCAO was reduced by preconditioning animals by a 30-min MCAO

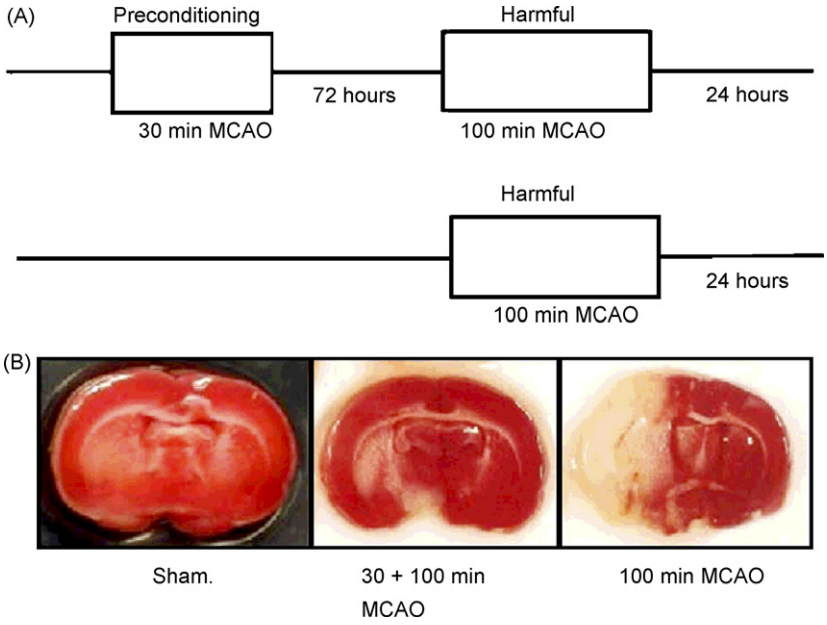

Fig. 1 – Preconditioning the brain with mild ischemia results in the brain being tolerant to harmful ischemia. (A) Model of *in vivo* ischemic tolerance. Tolerance is induced by subjecting animals to 30-min middle cerebral artery occlusion (MCAO) 72 h before a harmful, 100-min MCAO. Assessment of infarct is performed 24 h after the harmful ischemic insult. (B) Preconditioning reduces cerebral infarct after harmful focal ischemia. Animals were subjected to (1) sham, (2) 30-min MCAO followed by 72-h reperfusion and then a further 100-min MCAO, or (3) 100-min MCAO. Animals were killed 24 h after the last MCAO and infarcts were measured by triphenyltetrazolium chloride (TTC) staining.