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Research Report

Neuroprotection by neuregulin-1 in a rat model of permanent focal cerebral ischemia

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ABSTRACT

Neuregulin-1 (NRG-1) is a growth factor with potent neuroprotective capacity in ischemic stroke. We recently showed that NRG-1 reduced neuronal death following transient middle cerebral artery occlusion (tMCAO) by up to 90% with an extended therapeutic window. Here, we examined the neuroprotective potential of NRG-1 using a permanent MCAO ischemia (pMCAO) rat model. NRG-1 reduced infarction in pMCAO by 50% when administered prior to ischemia. We previously demonstrated using gene expression profiling that pMCAO was associated with an exaggerated excitotoxicity response compared to tMCAO. Therefore, we examined whether co-treatment with an inhibitor of excitotoxicity would augment the effect of NRG-1 following pMCAO. Both NRG-1 and the N-methyl-D-aspartate (NMDA) antagonist MK-801 similarly reduced infarct size following pMCAO. However, combination treatment with both NRG-1 and MK-801 resulted in greater neuroprotection than either compound alone, including a 75% reduction in cortical infarction compared to control. Consistent with these findings, NRG-1 reduced neuronal death using an *in vitro* ischemia model and this effect was augmented by MK-801. These results demonstrate the efficacy of NRG-1 in pMCAO rat focal ischemia model. Our findings further indicate the potential clinically relevance of NRG-1 alone or as a combination strategy for treating ischemic stroke.

1. Introduction

The neuregulins are a family of multipotent growth factors that includes acetylcholine receptor inducing activities (ARIAs), glial growth factors (GGFs), heregulins and neu differentiation factors (NDFs) (Falls et al., 1993; Ho et al., 1995; Holmes et al., 1992; Marchionni et al., 1993; Wen et al., 1992). A number of recent reports from our laboratory and others have shown that administration of NRG-1 reduces delayed ischemic cortical damage following transient middle cerebral artery occlusion (tMCAO) when administered before the onset of ischemia in rats (Guo et al., 2006; Shyu et al., 2004; Xu et al., 2004) or after tMCAO with an extended therapeutic window (Xu et al., 2006). The neuroprotective effects of the single administration of NRG-1 were seen up to 2 weeks following treatment. NRG-1 was neuroprotective if administered either before or 13.5 h after transient MCAO and resulted in a significant improvement of functional

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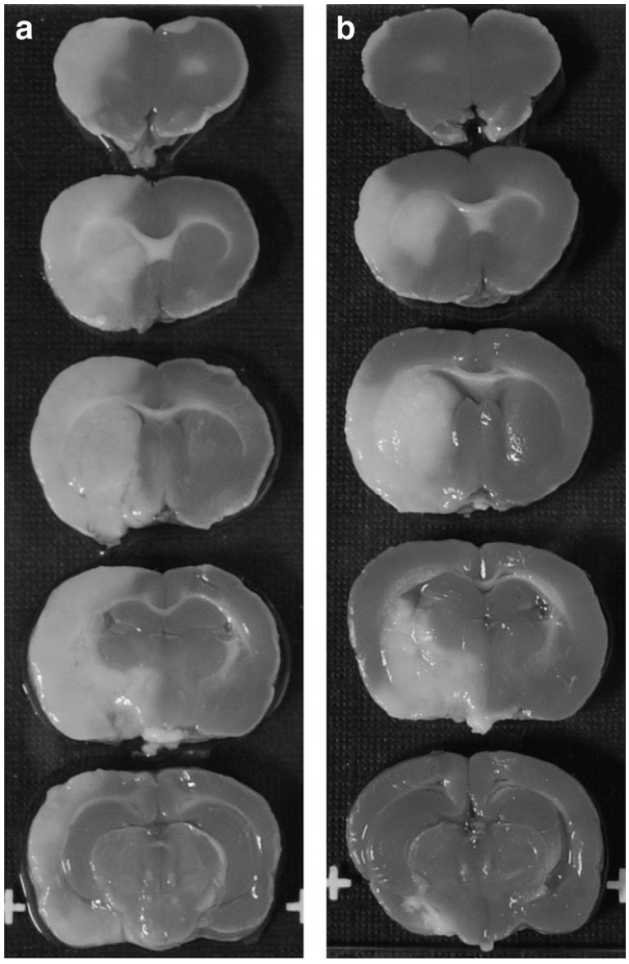

Fig. 1 – NRG-1 treatment reduces pMCAO-induced brain infarction. Representative TTC stained brain sections are shown where rats were injected with vehicle (a; $n=5$) or NRG-1 (b; $n=5$) before pMCAO. Animals were killed 24 h later and the brains were sliced into 2 mm sections and stained with 2,3,5-triphenyltetrazolium chloride (TTC). Infarct volumes in brains from vehicle and NRG-1 treated animals are shown in the graph (c). Values are presented as mean ± SD; * denotes significant difference from respective vehicle treated animals ($P<0.01$).

neurological outcome. NRG-1 also prevented glial activation, apoptosis and pro-inflammatory gene expression, further suggesting a role for NRG-1 in preventing delayed neuronal death following ischemia.

Many stroke investigators consider permanent MCAO (pMCAO) more ideal than tMCAO as a model for human stroke (STAIR, 1999). Therefore, in this study, we investigated the therapeutic potential of NRG-1 in the pMCAO model. Our findings demonstrated that NRG-1 is a potent neuroprotectant in pMCAO. We also showed that simultaneous NRG-1 administration and inhibition of glutamate excitotoxicity provided enhanced neuroprotection compared to either agent alone. These findings may result in the development of novel therapeutic strategies for the treatment of stroke.

2. Results

2.1. *NRG-1 reduced neuronal damage and improves neurological outcome following MCAO*

Rats were treated with NRG-1 immediately before pMCAO and sacrificed after 24 h. Fig. 1 illustrates a typical TTC staining of brain sections treated with vehicle or NRG-1 prior to pMCAO. Compared to control (Fig. 1a), pre-treatment with NRG-1 drastically reduced infarct volume after pMCAO (Fig. 1b). Infarct volume in the vehicle treated animals was 216.8 ± 25.0 mm^3. NRG-1 reduced the total infarct volume by 47.2% (Fig. 2).

The relative reduction in cortical and subcortical neuronal death was calculated. The infarct volume of control animals represented 54.3% of the total size of the ipsilateral hemisphere. The majority of the injury was localized to cortical brain regions. Treatment with NRG-1 resulted in an infarct that was reduced to represent only 26% of the total hemisphere; a 51% reduction in infarct size. NRG-1 treatment reduced the percentage of infarction in the cerebral cortex by 57% and by 41% in subcortical regions (Fig. 3).

2.2. *Combination treatment with NRG-1 and MK-801 prevents infarction following pMCAO*

We previously demonstrated that administration of NRG-1 prior to tMCAO prevented neuronal death by up to 90% following ischemia and reperfusion (Xu et al., 2005a, 2004). However, no further protection was conferred in the pMCAO model even when a twofold higher dose of NRG-1 was administered (data not shown). It is plausible that NRG-1 was not equally effective in the pMCAO model due to additional mechanisms that may be involved in pMCAO that are not available to NRG-1 treatment. Previous results from our laboratory using EASE software (Ford et al., 2006) showed that tMCAO/reperfusion was associated with apoptotic cell death and inflammation, which have been shown to be blocked by NRG-1 (Guo et al., 2006; Xu et al., 2005b, 2004). EASE identified

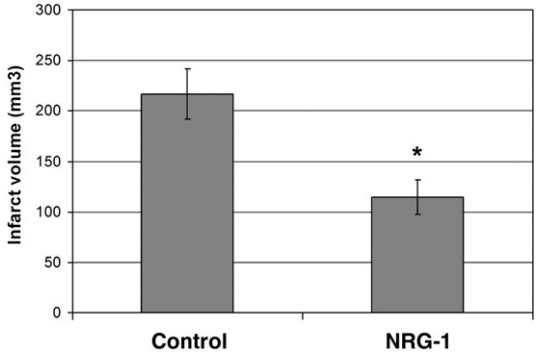

Fig. 2 – NRG-1 treatment reduces pMCAO-induced brain infarction. Infarct volumes in brains from vehicle and NRG-1 treated animals are shown in the graph. Values are presented as mean ± SD; * denotes significant difference from respective vehicle treated animals ($P<0.01$).