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Research Report

Protection of the brain following cerebral ischemia through the attenuation of PARP-1-induced neurovascular unit damage in rats

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ABSTRACT

Cerebral ischemia is a major health crisis throughout the world, and the currently available thrombolytic therapy is unsatisfactory. Cell death following cerebral ischemia is mediated by a complex pathophysiological interaction of various mechanisms. During an ischemic insult, not only neurons but all of the components of the neurovascular unit, such as glia, endothelia, pericytes and basal membranes, are destroyed. Previous studies have shown that excessive stimulation of poly (ADP-ribose) polymerase (PARP-1) is crucial for cerebral injury after ischemic insult, which is an important cause of cell death in all cell types within the neurovascular unit. To investigate whether PARP-1 plays an important role in protecting the neurovascular unit following cerebral ischemia, we evaluated neurobehavioral deficits, PARP-1 activity, blood brain barrier (BBB) disruption and neurovascular unit deficits using Western blot analysis, TTC staining and electron microscopy in an MCAO rat model. The results revealed that PARP-1 enzymatic activity was dramatically increased after ischemia. Inhibition of PARP-1 significantly reduced the extent of both cerebral infarction and edema, improved neurological scores, and attenuated the damage to the neurovascular unit in cerebral ischemia. Collectively, these findings demonstrate that the down-regulation of PARP-1 activity contributes to reducing post-ischemic brain damage via protection of the neurovascular unit.

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1. Introduction

Cerebral ischemia is the third leading cause of death throughout the world (Lloyd-Jones et al., 2009). Therapeutic strategies aimed at attenuating brain injury after ischemic stroke have been a major focus for the past 40 years. However, none of the many neuroprotective strategies studied in clinical experiments have produced the expected clinical outcomes (O''Collins et al., 2006; Savitz and Fisher, 2007). The classic therapeutic approach, known as the "thrombolytic approach," consists of rapid recovery of the occluded blood to the ischemic region, thus attenuating the neurological damage (Lo et al., 2003). The only drug therapy available in clinical settings is recombinant tissue plasminogen activator (rt-PA). However, due to the narrow therapeutic window and hemorrhagic complications, only a minority of patients receive rt-PA therapy. Further analyses have documented that the key reason for these failures may be that only a single therapeutic target is pursued and that the interactions among the various components of the neurovascular unit (NVU) are neglected (Xue et al., 2013). Indeed, to obtain substantial and long-lasting neuroprotection, not only neurons but all of the components of the NVU, such as glia, endothelia, pericytes and basal membranes, should be targeted during an ischemic insult (Iadecola et al., 2006). Hence, there is an urgent need to develop effective neuroprotective agents that combine multi-target and multi-level therapy. To achieve this goal, an increasing number of strategies for ischemic neuroprotection through various targets have been proposed and evaluated in preclinical settings. However, these targets, which include all components of the NVU, increase the already substantial complexity and decrease the feasibility of obtaining ischemic neuroprotection by pharmacological approaches. Recent comprehensive findings from various cerebral ischemia models have demonstrated that the direct activation of poly (ADP-ribose) polymerases (PARPs), especially PARP-1, which is a key mediator of ischemic injury, plays an important role in neuroprotection against cerebral necrosis and apoptosis (Chiarugi, 2005; Kauppinen et al., 2009; Matsuura et al., 2011; Moroni, 2008). Therefore, we speculate that the regulation of PARP-1 activity may be one of the most efficacious means of protecting the NVU currently available.

PARP-1 is the most abundant of several PARP family members and is activated by binding to DNA strand breaks under many oxidative stress-related conditions (Castri et al., 2014). The hyperactivation of PARP-1 after cerebral ischemia occurs not only in neurons but also in astrocytes, microglia, endothelia, pericytes and infiltrating leukocytes, and this effect contributes to cell death through a process involving excessive ATP and NAD^+ depletion, the release of inflammatory factors and free radicals, and apoptosis-inducing factor (AIF) translocation from the mitochondria to the nucleus (Castri et al., 2014; Kauppinen et al., 2009; Moroni, 2008). Previous studies have demonstrated that the inhibition of PARP-1 activation by PARP-1 gene knockout or pharmacological inhibitors (PJ-34, 3-AB and MP-124) following focal cerebral ischemia could drastically reduce the ischemia-induced neurological damage (Hamby et al., 2007; Matsuura et al., 2011; Shimizu et al., 2013; Singh et al., 2014; Yap et al., 2008). It has been suggested that PARP-1 may be a crucial cofactor of NVU disruptions leading to cerebral injury.

Therefore, in the present study, we investigated the protective role of PARP-1 down-regulation within the NVU following cerebral ischemia. First, we established a model of middle cerebral artery occlusion (MCAO); then, we evaluated whether the down-regulation of PARP-1 could ameliorate neurobehavioral deficits, blood brain barrier (BBB) disruptions and synaptic deficits using small hairpin RNA (shRNA), TTC staining, Western blot analysis and electron microscopy.

2. Results

2.1. PARP-1 activation was induced by cerebral ischemia in rats

During ischemia, PARP-1 is activated by DNA breaks and cleaved into two fragments that measure 24 and 89 kDa. Thus, we chose PARP-1 cleavage (89 kDa) as a marker of PARP-1 activity. The protein expression of PARP-1 cleavage was quantified by Western blotting at 1 h, 3 h, 6 h, 24 h and 3 d post-MCAO (Fig. 1). Densitometric analysis demonstrated a significant increase in protein levels in the rats at 1 h and 3 h post-MCAO compared with those in sham animals ($p<0.01$, $p<0.01$, Fig. 1). Compared with the 1 h group, PARP-1 expression was drastically reduced in rats 6 h after MCAO ($p<0.01$, $p<0.01$).

2.2. PARP-1 expression in rats was suppressed by treatment with LV-PARP1-shRNA

LV-PARP1-shRNA was successfully transfected into more than 70% of neurons 72 h after infection at an MOI of 5 (Fig. 2). ShRNA1, shRNA2 and shRNA3 all suppressed PARP-1 expression notably ($p<0.01$, Fig. 2), while no knock-out effect was observed in the negative control group (LV-control-

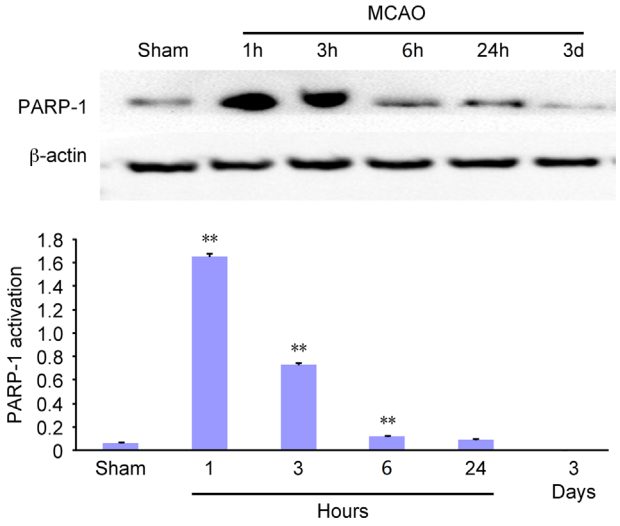

Fig. 1 – PARP-1 activity in cerebral ischemic rats. (A) A Western blot shows the expression of PARP-1 cleavage at 1 h, 3 h, 6 h, 24 h and 3 d post-MCAO. (B) Quantitative analysis of PARP-1 cleavage. PARP-1 is significantly increased in rats 1 h post-MCAO (similar to the 3 h group) compared to the sham animals. $n=3$, $^{**}p<0.01$ vs the control group.