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Research Report

PARP inhibition attenuates early brain injury through NF-κB/MMP-9 pathway in a rat model of subarachnoid hemorrhage

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ABSTRACT

Poly (ADP-ribose) polymerases (PARPs) play an important role in a range of neurological disorders, however, the role of PARP in early brain injury after subarachnoid hemorrhage (SAH) remains unclear. This study was designed to explore the role and the potential mechanisms of PARP in early brain injury after SAH. Eighty-nine male SD rats were randomly divided into the Sham group, SAH+Vehicle group and SAH+PARP inhibitor (PJ34) group. An endovascular perforation model was used to induce SAH in rats. PJ34 (10 mg/kg) or vehicle (0.9% NaCl) was intraperitoneally administered at 5 min and 8 h after SAH induction. Mortality, SAH grades, neurological function, evans blue extravasation, brain edema, immunofluorescence staining and western blotting were performed. PJ34 reduced BBB permeability and brain edema, improved neurological function and attenuated neuronal cell death in the rat model of SAH. Moreover, PJ34 inhibited the nuclear translocation of NF-κB, decreased the expression of the proinflammatory cytokines IL-1ß, IL-6 and TNF-α, reduced the expression of MMP-9, prevented the degradation of tight junction proteins, and decreased microglia activation. These data indicated that PARP inhibition through PJ34 might be an important therapeutic drug for SAH.

1. Introduction

Subarachnoid hemorrhage (SAH) is a life-threatening acute cerebrovascular event that occurs primarily in working-aged individuals. Approximately 20% of patients suffering from SAH suddenly died (Korja and Kaprio, 2015), and 50% of SAH survivors presented the cognitive deficits and chronic functional decline (Connolly et al., 2012). Previously, delayed vasospasm was regarded as the most important cause of poor outcome after SAH (Dorsch, 1995). However, clinical trials based on this strategy did not show a reduction in mortality and neurological deficits after SAH (Macdonald et al., 2011, 2012). Recently, early brain injury (EBI) has been recognized and been considered as the primary cause of mortality in SAH patients (Helbok et al., 2015). The immediate physiological derangement includes the following features: increased intracranial pressure (ICP), decreased cerebral blood flow (CBF), and global cerebral ischemia. These events initiate secondary injuries such as blood brain barrier (BBB) disruption, inflammation and cell death. Consequently, pharmaceutical interventions for the treatment of EBI might be helpful for the prognosis of SAH patients.

Poly (ADP-ribose) polymerases (PARPs) catalyze the transfer of ADP-ribose units from nicotinamide adenine dinucleotide (NAD^+) to substrate proteins (Moroni, 2008). These enzymes play key roles in DNA repair, mitotic progression and gene expression. The hyperactivation of PARP results in the marked depletion of NAD^+ and ATP stores which might lead to necrosis (Berger, 1985). In addition, PARP activation directly induces the nuclear translocation of apoptosis-inducing factor (AIF), resulting in caspase-independent cell death (Li et al., 2010). Moreover, a growing body of studies have indicated that PARP mediates gene expression through interactions with transcription factors, particularly, nuclear factor-κB (NF-κB) (Hassa et al., 2003; Kameoka et al., 2000). PARP-dependent-NF-κB triggers pro-inflammatory gene expression and microglia activation upon the release of TNF-α, IL-1ß and matrix metalloproteinases-9 (MMP-9) (Chiarugi and Moskowitz, 2003; Ullrich et al., 2001), which are responsible for the disruption of the capillary basal lamina, the opening of the blood-brain

Abbreviations: SAH, subarachnoid hemorrhage; EBI, early brain injury; ICP, intracranial pressure; CBF, cerebral blood flow; BBB, blood brain barrier; PARP, poly (ADP-ribose) polymerases; NAD, nicotinamide adenine dinucleotide; AIF, apoptosis-inducing factor; MMP-9, matrix metallopeptidase 9; NF-κB, nuclear factor-κB; TUNEL, terminal deoxynucleotidyl transferase-mediated.

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barrier (BBB), the infiltration of leukocytes and further brain edema. These genes are strongly reduced in PARP knockout rats or following the administration of PARP inhibitors (Ha et al., 2002; Haddad et al., 2006; Kauppinen et al., 2009).

In the present study, we hypothesized that PARP-dependent neuroinflammation plays key roles in BBB disruption and brain edema in EBI following SAH, and the administration of PJ34 (N-(6-oxo-5,6-dihydrophenanthridin-2-yl)-N,N-dimethylacetamide), a PARP inhibitor, attenuates neuroinflammation-induced BBB disruption and brain edema, and improves neurological deficits after experimental SAH.

2. Results

2.1. Mortality and SAH grade

The observed mortality rates were sham 0% (0/23), SAH+Vehicle 32.3% (11/34) and SAH+PJ34 28.1% (9/32). The mortality rates were not significantly different between the SAH groups ($P > 0.05$). Similarly, no significantly differences in the SAH grades were observed among all SAH groups ($P > 0.05$, Fig. 1(A)).

2.2. Neurological scores

The neurological scores were significantly worse in the SAH+Vehicle group ($P < 0.05$, Fig. 1(B)); however, SAH animals that received PJ34 demonstrated significantly improved neurological performances at 24 h after SAH-induction ($P < 0.05$, Fig. 1(B)).

2.3. BBB permeability and brain water content

The SAH+Vehicle rats showed that marked extravasation of Evans blue dye into the left hemispheres at 24 h post-SAH ($P < 0.05$, Fig. 1(C)). PJ34 treatment significantly reduced the amount of Evans blue dye extravasation in the left hemispheres ($P < 0.05$, Fig. 1(C)). Similarly, the brain water content in the left hemispheres was significantly increased in the SAH+Vehicle group compared with the sham group at 24 h post-SAH ($P < 0.05$, Fig. 1(D)). PJ34 treatment significantly reduced the water content in the left hemispheres ($P < 0.05$, Fig. 1(D)).

2.4. PJ34 reduced MMP-9 expression and prevented degradation of occludin and claudin-5

Western blot analysis was used to quantify the expression of MMP-9, occludin and claudin-5 in the left brain hemisphere at 24 h after surgery. MMP-9 was significantly higher in the SAH+Vehicle group compared with the sham group ($P < 0.05$, Fig. 2(A) and (B)), and this effect was reversed after PJ34 administration ($P < 0.05$, Fig. 2(A) and (B)). Occludin and claudin-5 were significantly decreased in the SAH+Vehicle group compared with the sham group ($P < 0.05$, Fig. 2(A), (C) and (D)). PJ34 treatment significantly prevented the degradation of occludin and claudin-5 compared with the SAH+Vehicle group ($P < 0.05$, Fig. 2(A), (C) and (D)).

2.5. PJ34 reduced proinflammatory cytokines IL-1ß, IL-6 and TNF-α expression

Western blot analysis revealed that the expression of IL-1ß, IL-6

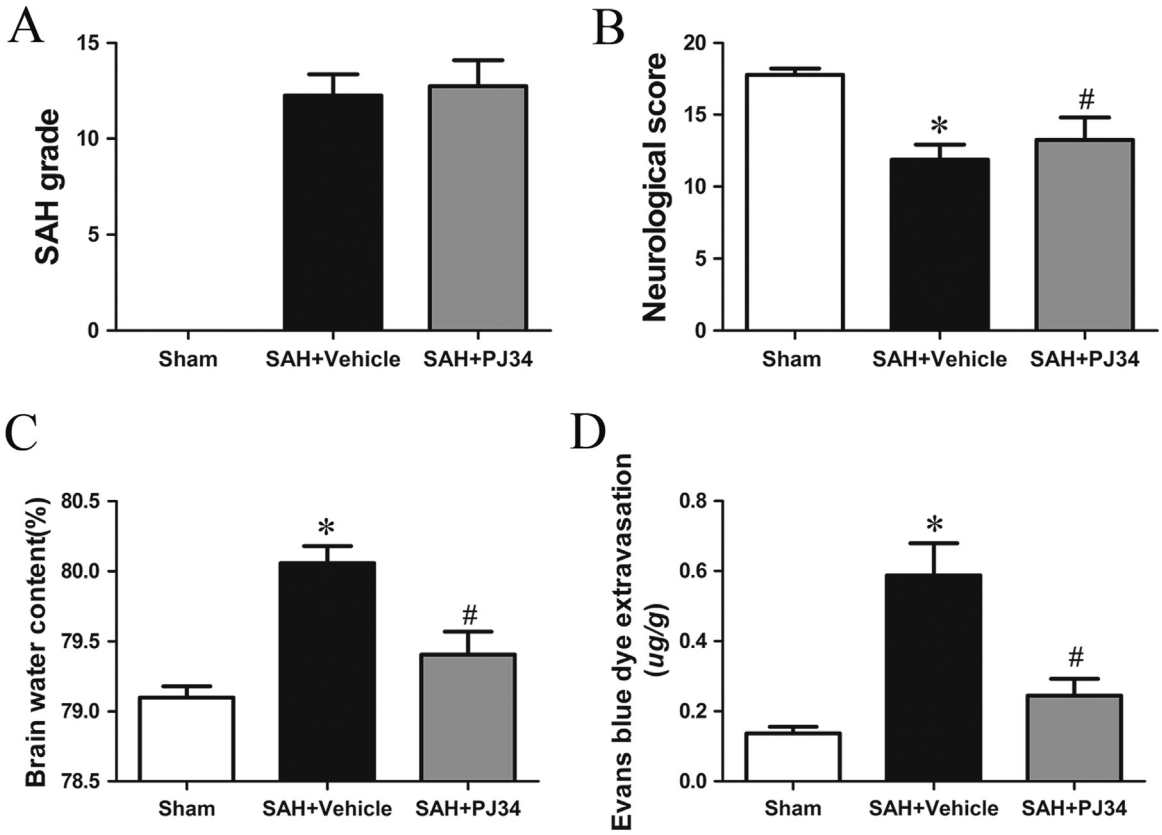

Fig. 1. SAH grade, neurological scores, brain water content and evans blue dye extravasation at 24 h after SAH. (A) SAH severity. The bars represent the mean ± SD. n=23. (B) Neurological scores. The bars represent the mean ± SD. n=23. *p < 0.05 vs sham, #p < 0.05 vs SAH+Vehicle. (C) Brain water content. The bars represent the mean ± SD. n=6. *p < 0.05 vs sham, #p < 0.05 vs SAH+Vehicle. (D) Evans blue dye extravasation. The bars represent the mean ± SD. n=6. *p < 0.05 vs sham, #p < 0.05 vs SAH+Vehicle.