Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Recombinant osteopontin attenuates experimental cerebral vasospasm following subarachnoid hemorrhage in rats through an anti-apoptotic mechanism

Junchi He, Mindi Liu, Zhen Liu, Liangsheng Luo[*,1]

Department of Neurosurgery, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, China

ARTICLE INFO

Article history:
Accepted 6 March 2015
Available online 14 March 2015

Keywords:
Osteopontin
Subarachnoid hemorrhage
Cerebral vasospasm
Apoptosis

ABSTRACT

Cerebral vasospasm (CVS) is an important pathological process following subarachnoid hemorrhage (SAH). Osteopontin (OPN), a pleiotropic extracellular glycoprotein, has been reported to be able to induce MKP-1 in the spastic cerebral arteries and prevent vasospasm after SAH. The purpose of this study was to investigate the protective effects of recombinant OPN (r-OPN) on CVS following SAH and the underlying mechanisms associated with its anti-apoptotic effect. Eighty male Sprague Dawley rats (weighing 300–375 g) were randomly assigned to four groups: (1) sham+vehicle (n=20), (2) SAH+vehicle (n=20), (3) SAH+OPN0.03 (0.03 μg) (n=20), (4) SAH+OPN0.1 (0.1 μg) (n=20). The double injection model of cisterna magna was performed on day 0 and 48 h after the first induction. r-OPN was administered intraventricularly nearly 30 min after the first SAH. After neurological score assessment, rats were sacrificed 72 h after the first SAH. The cross-sectional area and thickness of basilar arteries (BA) were measured under Hematoxylin–eosin (H&E) staining. Endothelial cell apoptosis was identified by terminal deoxynucleotidyl transferase mediated nick end labeling (TUNEL) staining. Immunohistochemistry was used to assess the expression of p-Akt and cleaved caspase-3 in BA. Western blot analysis was applied to evaluate the expression of p-Akt, cleaved caspase-3, Bax and Bcl-2 in BA. r-OPN improved neurological scores and attenuated vasospasm. r-OPN significantly reduced expression of cleaved caspase-3 and Bax in BA following SAH, and increased the level of p-Akt and Bcl-2, coupled with reduced apoptosis of endothelial cell in BA. These results demonstrate that r-OPN can attenuate vasospasm after SAH through a suppressed apoptotic response, which may provide a novel therapeutic target for cerebral vasospasm.

*Corresponding author.
E-mail addresses: iverjun@hotmail.com (J. He), lsluo@sohu.com (L. Luo).
[1]Postal address: 68 Changle Road, Qinhuai District, Nanjing, Jiangsu, China.

http://dx.doi.org/10.1016/j.brainres.2015.03.015
0006-8993/

1. Introduction

Subarachnoid hemorrhage (SAH) is a common and devastating cerebrovascular disease, mostly caused by intracranial aneurysm. Cerebral vasospasm (CVS), the delayed narrowing of arteries at the base of the brain, has been thought to be the leading cause of high morbidity and mortality following SAH. Researches have been focused on CVS over the last few decades, whereas treatment strategies dealing with CVS have failed to improve outcomes (Ciurea et al., 2013). Therefore, a new therapeutic approach is warranted to attenuate CVS and improve prognosis. The pathogenesis of CVS being unclear, apoptosis of endothelial cells has been shown to promote the development of vasospasm (Zhou et al., 2005).

Osteopontin (OPN) is a secreted pleiotropic extracellular matrix glycoprotein with roles in a variety of physiological and pathological processes, including tissue remodeling, fibrosis, cell migration, anti-apoptotic processes, and inflammation (Chen et al., 2011b; Higashikawa et al., 2007). Recent studies have been focused on the role of OPN in central nervous system. Administration of recombinant OPN (r-OPN) attenuated intracerebral hemorrhage-induced brain injury by suppressing matrix metalloproteinase-9 (MMP-9) activity via inhibiting inducible nitric oxide synthase (iNOS) upregulation (Wu et al., 2011). Also, r-OPN has been demonstrated to prevent early brain injury (EBI) and stabilize blood brain barrier disruption by inhibiting NF-κB dependent MMP-9 induction (Suzuki et al., 2010a). Moreover, r-OPN treatment prevented cerebral vasospasm by inducing an endogenous MAPK inhibitor MAPK phosphatase (MKP)-1 in the endovascular perforation model in rats (Suzuki et al., 2010b). It is unclear, however, whether there are other mechanisms involving the protective effect of OPN against CVS, such as anti-apoptotic effect. The present study was aimed to examine the effects of r-OPN on CVS in a double injection model of rats and to demonstrate its anti-apoptotic mechanisms alleviating CVS.

2. Results

2.1. Motality

The motality at 72 h was none (0 of 20) in sham+vehicle group, 20.0% (5 of 25) in SAH+vehicle group, 16.7% (4 of 24) in SAH+OPN0.03 group, 13.0% (3 of 23) SAH+OPN0.1 group. No significant difference was found in mortality between the SAH groups.

2.2. r-OPN improved neurological scores

Neurological scores are shown in Fig. 1. Compared with Sham+vehicle group, neurological scores in the SAH+vehicle group were significantly poorer between 24 h and 72 h ($p<0.01$). High dose r-OPN significantly improved scores compared with the SAH+vehicle group at 48 h and 72 h ($p<0.01$). Neurological scores showed no significant difference between the SAH+OPN0.03 group and the SAH+vehicle group ($p>0.05$).

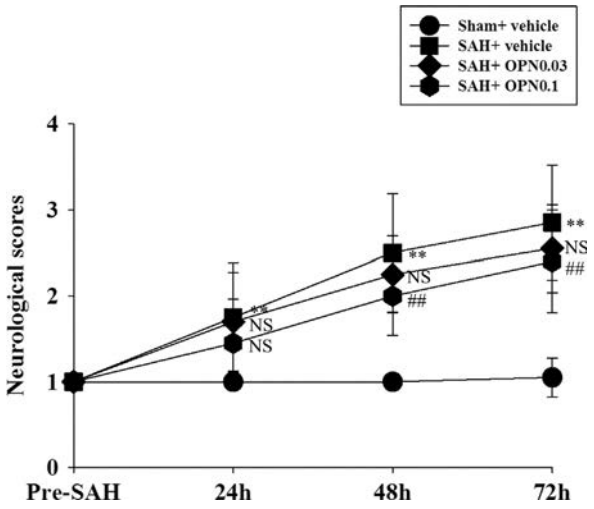

Fig. 1 – Effect of r-OPN treatment on neurological scores after SAH. Neurological scores in the SAH+vehicle group were significantly poorer than those in the Sham+vehicle group between 24 h and 72 h. r-OPN0.1 treatment significantly improved scores compared with the SAH+vehicle group at 48 h and 72 h. ** $p<0.01$ versus Sham+vehicle group, NS $p>0.05$ versus SAH+vehicle group, ## $p<0.01$ versus SAH+vehicle group.

2.3. r-OPN attenuated CVS following SAH

The mean cross-sectional area and thickness of BA each group are shown in Fig. 2. The mean cross-sectional area of BA significantly decreased in the SAH+vehicle group compared with the sham+vehicle group ($p<0.01$), while it was significantly increased by administration of 0.1 μg r-OPN ($p<0.01$) and 0.03 μg r-OPN ($p<0.05$) respectively compared with the SAH+vehicle group. The thickness of the vessel walls significantly increased in the SAH+vehicle group compared with that in the sham+vehicle group ($p<0.01$). Both high dose and low dose r-OPN significantly decreased the thickness respectively compared with the SAH+vehicle group ($p<0.01$).

2.4. r-OPN reduced endothelial apoptosis in BA after SAH

TUNEL staining of BA in each group is shown in Fig. 3. Few TUNEL-positive apoptotic cells were detected in BA in the sham+vehicle group. In the SAH+vehicle group, the apoptotic index in BA endothelial cells was significantly elevated as compared to that in the sham+vehicle group ($p<0.01$), and was significantly declined in both SAH+OPN0.03 and SAH+OPN0.1 groups compared with that in the SAH+vehicle group ($p<0.01$).

2.5. Effects of r-OPN on p-Akt, cleaved caspase-3, Bax and Bcl-2 in basilar arteries after SAH

Western blot expressions of p-Akt, cleaved caspase-3, Bax and Bcl-2 are shown in Fig. 4. Western blot analysis revealed a significant up-regulation of p-Akt in BA in the SAH+OPN0.1 group compared with the sham+vehicle group ($p<0.01$) and the SAH+vehicle group ($p<0.01$) respectively. Also, an