Neuroscience Letters 552 (2013) 81–86

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

The role of the sonic hedgehog signaling pathway in early brain injury after experimental subarachnoid hemorrhage in rats

Tao Li[a,b], Jie Zhang[c], Rong-Yao Liu[b], Zhi-Gang Lian[b], Xiao-Lin Chen[a], Li Ma[a], Hao-Min Sun[a], Yuan-Li Zhao[a,*]

[a] Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, PR China
[b] The First Hospital of Dalian Medical University, Dalian 116021, PR China
[c] Center of Reproductive Medicine, Dalian Obstetrics and Gynecology Hospital, Dalian 116021, PR China

HIGHLIGHTS

- The mRNA and protein levels of Shh, Patched1, and Gli-1 were up-regulated in the cortex after SAH.
- Cyclopamine increased the MDA and decreased the SOD and GSH-Px enzyme activities in brain.
- Cyclopamine increased cell apoptosis, upregulated the Bax, and downregulated the Bcl-2.
- In the cyclopamine-treated group, early brain damages were significantly aggravated.

ARTICLE INFO

Article history:
Received 19 May 2013
Received in revised form 9 July 2013
Accepted 27 July 2013

Keywords:
Subarachnoid hemorrhage
Sonic hedgehog pathway
Cyclopamine
Early brain injury

ABSTRACT

Previous studies have demonstrated that the sonic hedgehog (Shh) pathway plays a neuro-protective role. However, whether the Shh pathway is induced by subarachnoid hemorrhage (SAH) has not been investigated. We sought to investigate Shh activation in the cortex in the early stage of SAH, and assessed the effect of cyclopamine (a specific inhibitor of the Shh pathway) on Shh pathway regulation and evaluated the impact of cyclopamine on SAH. We found that the Shh pathway was up-regulated in the cortex after SAH, and that blocking the Shh pathway increased cell apoptosis. Early brain damages, including brain edema, blood-brain barrier impairment, and cortical apoptosis were significantly aggravated following with cyclopamine treatment compared with vehicle treatment. Our results suggest that the Shh pathway should be activated in the brain after SAH, and plays a beneficial role in SAH development, possibly by inhibiting cerebral oxidative stress through induction of antioxidant and detoxifying enzymes.

1. Introduction

Experimental and clinical studies have shown that the acute symptoms of subarachnoid hemorrhage (SAH) are attributed to increased intracranial pressure, decreased cerebral perfusion pressure and the resulting ischemia [1,2]. Despite the potentially devastating consequences of cerebral vasospasm and intensive investigations over the past 4 decades, the detailed pathogenesis of cerebral vasospasm is incompletely understood, and no optimal treatments have yet been established. Many strides have been made to understand the common secondary complications that occur after SAH, especially focusing on complications that occur early on, often known as early brain injury (EBI) [1,3–5].

The hedgehog protein family consists of Shh, Indian hedgehog (Ihh), and desert hedgehog (Dhh) [6]. By binding to its receptor Patched, Shh releases the transmembrane protein Smoothened (Smo) which binds to Patched, and activates the transcription factor glioma associated oncogene (Gli). Gli translocates to the nucleus and thereby regulates the expression of a number of target genes that control cell growth, survival, and differentiation [7–9]. Recent reports suggested that Shh is implicated in the development of neurodegenerative diseases and brain injury [10–13]. However, until now, no study has investigated the potential contribution of the Shh pathway to SAH-induced EBI. The aim of the current study was to evaluate the cortical activity of the Shh pathway following SAH and determine the potential role of the Shh pathway in the development of EBI.

2. Materials and methods

For details of perfusion-fixation, western blotting, qRT-PCR, immunohistochemistry, measurement of MDA,SOD, and GSH-Px activities, brain water content, blood-brain barrier (BBB)

* Corresponding author. Tel.: +86 13 583108758; fax: +86 10 67096611.
E-mail address: zyltg2013@163.com (Y.-L. Zhao).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.07.042

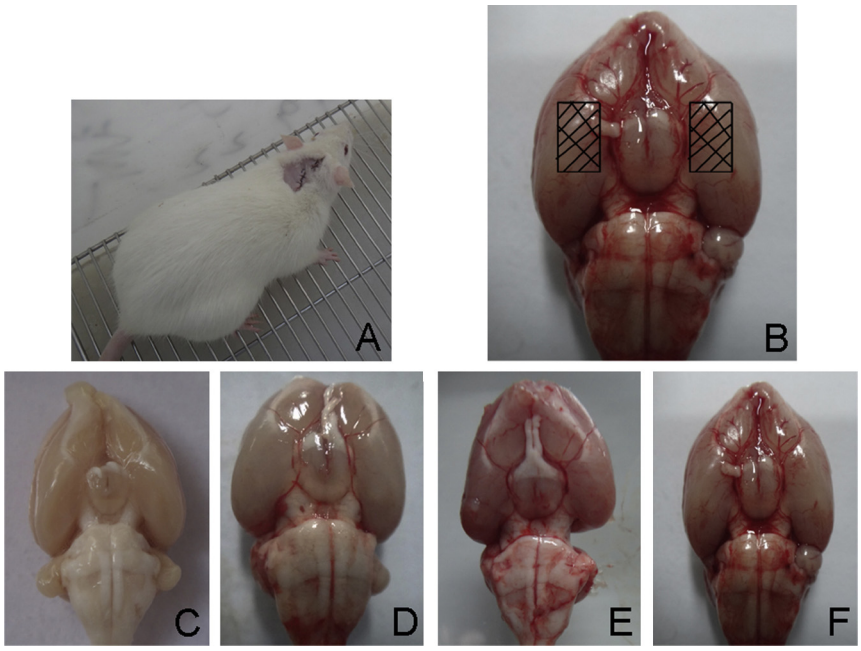

Fig. 1. General observations in rats after SAH (A). Schematic representations of the areas taken for analysis (B). Ventral view of typical brains from the control (C), SAH (D), SAH + vehicle (E), and SAH + cyclopamine (F) groups.

permeability, TUNEL staining, seeing Supplemental Materials and Methods.

2.1. Animals

Male Wistar rats (250–300 g) were purchased from the Animal Center of the Chinese Academy of Sciences, Beijing, China. The rats were housed in temperature and humidity controlled animal quarters with a 12-h light/dark cycle. All procedures were approved by the Institutional Animal Care Committee and were conducted in accordance with the Guide for the Care and Use of Animals of the National Institutes of Health.

2.2. Rat SAH model

Rats were anesthetized with pentobarbital (40 mg/kg, IP), and allowed to breathe spontaneously. Aided by a surgical microscope, a small suboccipital incision was made, exposing the arch of the atlas, the occipital bone, and the atlantooccipital membrane. With a 27-gauge needle, the atlanto-occipital membrane was tapped carefully into the cisterna magna. Fresh autologous nonheparinized blood (0.3 mL) from the femoral artery was injected over a period exceeding 2 min. After the blood injection, the hole was sealed with an absorbable sponge to prevent a fistula and the wound was immediately sutured. The rats were then placed in a head-down prone position at a 30° angle for 30 min to hold the blood in the basal cisterns. In control animals, the same technique was applied by injection of sterile saline instead of blood.

2.3. Experimental design

The experimental groups consisted of a control group ($n = 30$), an SAH group ($n = 30$), an SAH + vehicle group ($n = 15$), and an SAH + cyclopamine group ($n = 15$). The animals in the SAH + cyclopamine group received an intraperitoneal injection of cyclopamine (10 mg/kg) [14] (Sigma–Aldrich, St. Louis, MO) at 0, 12, 24, and 36 h after the blood injection and animals in the SAH + vehicle group received equal volumes of vehicle (30% (w/v) 2-hydroxypropyl-b-cyclodextrin (HPBCD) in 0.1 M sodium citrate phosphate buffer, pH 3.0) at the corresponding time points. The animals in control and SAH groups were killed at 12, 24, and 48 h post-SAH, respectively ($n = 5$ for each group). The rats in each group were exsanguinated and decollated. Abrain sample was removed and rinsed in 0.9% normal saline several times to wash away blood and blood clots. Then, the tissue was immediately frozen in liquid nitrogen immediately for molecular biological and biochemical experiments. All the remaining rats were killed at 48 h post-SAH. Five rats in each group were sacrificed by the fixation perfusion method. The cortex was taken for immunohistochemical staining and terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL). Another 5 rat's brain samples were immediatedly frozen in liquid nitrogen immediately for molecular biological and biochemical experiments. The other 5 rats were used for test for BBB impairment.

2.4. Statistical analysis

Data was described as the mean ± SD, and analyzed by Student's two-tailed t-test. The limit of statistical significance was $P < 0.05$. Statistical analysis was done with SPSS/Win11.0 software (SPSS, Inc., Chicago, Illinois).

3. Results

3.1. General observations

There were no significant differences in body weight, temperature, or injected arterial blood gas data among the experimental groups (data not shown). As shown in Fig. 1, the rats in the SAH, SAH + vehicle, and SAH + cyclopamine groups exhibited blood clots over the basal surface of the brainstem, and there were no significant differences among these 3 groups.

3.2. Shh pathway activity after SAH

Western blot and qRT-PCR analysis showed low levels of Shh, Patched1, and Gli1 in the control group (Fig. 2A, Supplemental Fig. 1). The Shh, Patched1, and Gli-1 expression were increased