Neuroscience Letters 543 (2013) 163–167

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

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

Progesterone attenuates early brain injury after subarachnoid hemorrhage in rats

Feng Yan, Qiang Hu, Jingyin Chen, Cheng Wu, Chi Gu, Gao Chen*

Department of Neurosurgery, The Second Affiliated Hospital of Zhejiang University School of Medicine, NO. 88 Jiefang road, Hangzhou 310009, China

HIGHLIGHTS

- We explored the effect of progesterone on rats subjected to subarachnoid hemorrhage.
- Progesterone significantly improved neurological deficits and reduced mortality.
- Progesterone can down-regulated caspase-3 expression and reduce cell apoptosis.
- Progesterone stabilized BBB and reduced brain edema may by down-regulating MMP-9.

ARTICLE INFO

Article history:
Received 8 February 2013
Received in revised form 3 March 2013
Accepted 6 March 2013

Key words:
Subarachnoid hemorrhage
Early brain injury
Progesterone
Apoptosis
Blood–brain barrier

ABSTRACT

Background and purpose: Although the neuroprotective effects of progesterone against early brain injury (EBI) after trauma have been demonstrated in several studies, whether progesterone reduces EBI after subarachnoid hemorrhage (SAH) remains unknown. In this study, we explored the effect of progesterone on cell apoptosis, stability of the blood–brain barrier (BBB), brain edema, and mortality in male Sprague-Dawley rats subjected to subarachnoid hemorrhage-induced EBI by endovascular perforation.
Method: Rats ($n = 66$) were randomly assigned to sham, SAH + vehicle, and SAH + progesterone groups. Progesterone (16 mg/kg) or an equal volume of vehicle was administered at 1 h, 6 h and 12 h after SAH. Mortality within 24 h, neurological scores, brain edema, Evans blue dye extravasation, cell apoptosis, and the expression of caspase-3 and matrix metalloproteinase (MMP)-9 were assayed after 24 h of SAH.
Result: Progesterone treatment significantly reduced mortality, brain edema, Evans blue dye extravasation, cell apoptosis, expression of caspase-3 and MMP-9, and improved neurological scores compared with the vehicle group.
Conclusion: Progesterone may reduce EBI after SAH by inhibiting cell apoptosis and stabilizing the BBB.

1. Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a serious condition with high mortality and morbidity. Delayed vasospasm arises in nearly 70% of aSAH patients 3–12 days after the rupture of the aneurysm [1,9] and is considered to be the cause of the poor prognosis of aSAH. Therefore, preventing delayed ischemic injury due to delayed vasospasm has been an active area of investigation for the treatment of aSAH. However, there is no established method to effectively prevent vasospasm and improve the outcome of aSAH [32], which may be because there is a dissociation between the presence of vasospasm and the poor clinical outcome of aSAH [1,9]. This dissociation is further supported by the fact that nimodipine improves the neurological outcome in aSAH patients but does not affect the angiographic vasospasm [2,5]. In contrast, clazosentan, an ET-1A antagonist, reduced vasospasm but failed to improve the neurological outcome in aSAH patients [20,22]. The early brain injury (EBI) after aSAH seems to be an important factor in the poor outcome of aSAH patients because cell (neurons, astrocytes and oligodendrocytes) death occurs within 24 h after aSAH [27], and the immediate destruction of the basal lamina induced by aSAH [15] leads to a progressive increase in brain blood brain barrier (BBB) permeability and edema [10,12]. In addition, the increase in extracellular glutamate, oxidative stress and inflammation also contribute to EBI after aSAH [12,29,36]. Therefore, reducing EBI may effectively lessen the intensity of delayed ischemic injury and improve the neurological outcome in aSAH patients. The neuroprotective effect of progesterone in EBI after trauma has been supported by numerous studies [18,21]. Progesterone is effective in decreasing cell death within 24 h after injury [7], reducing tissue damage and improving behavioral recovery following traumatic brain injury [33], stabilizing the BBB and reducing brain edema [24], and reducing lipid peroxidation, glutamate release and neuronal apoptosis [25]. However, the effect of progesterone on EBI after SAH remains unclear.

In the current work, we hypothesized that progesterone might reduce EBI after SAH and improve neurological

* Corresponding author. Tel.: +86 13805716226; fax: +86 057187784753.
E-mail address: d.chengao@163.com (G. Chen).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.03.005

outcomes by inhibiting cell apoptosis and stabilizing the BBB.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (280–300 g) were from the SLAC Laboratory Animal Co. Ltd (Shanghai, China) and were randomly assigned to three groups: the sham + vehicle group ($n = 22$) was subjected to a similar procedure as the SAH group but without perforation and received 22.5% 2-hydroxypropyl-β-cyclodextrin as the vehicle; the SAH + vehicle group ($n = 22$) was subjected to SAH and treated with the same volume of vehicle as the sham + vehicle group; the SAH+ progesterone group ($n = 22$) was subjected to SAH and treated with progesterone. All endpoints in this study were investigated at 24 hour after SAH. All experimental protocols were approved by the Ethics Committee for the Use of Experimental Animals in Zhejiang University.

2.2. Rat SAH model

The rat SAH monofilament puncture model was performed as previously described [26]. Briefly, after rats were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg), the carotid artery was dissected, and a blunted 4–0 monofilament nylon suture was placed in the external carotid artery and advanced through internal carotid artery to the junction of the middle cerebral artery and the anterior cerebral artery, where the filament perforated to create a SAH. In the sham surgery group, a similar procedure was performed without perforation. The severity of the SAH was quantified according to a previously published grading scale [35]. The scale was based on the amount of subarachnoid blood in 6 segments of basal cistern: grade 0, no subarachnoid blood; grade 1, minimal subarachnoid blood; grade 2, moderate blood with visible arteries; grade 3, blood clot covering all arteries within the segment. A total score ranging from 0 to 18 was obtained by adding the scores from all 6 segments.

2.3. Drug administration

Progesterone (P-0130; Sigma-Aldrich Co., St. Louis, MO) was dissolved in 22.5% 2-hydroxypropyl-β-cyclodextrin(H107; Sigma-Aldrich Co., St. Louis, MO, as previously described [16]. The initial injection of progesterone (16 mg/kg) was given intraperitoneally 1 h after SAH, and subsequent injections were given subcutaneously 6 h and 12 h after SAH. The sham + vehicle group and the SAH + vehicle group rats were injected identically with same volume of the vehicle (22.5% 2-hydroxypropyl-β-cyclodextrin) relative to body weight.

2.4. Evaluation of mortality and neurological deficits

Mortality was determined, and neurological deficits were evaluated after 24 h of SAH according to the scoring system of Garcia [11]. Briefly, spontaneous activity, symmetry in the movement of four limbs, forepaw outstretching, climbing, body proprioception, and response to vibrissae touch were tested. These six tests were each scored from 0 to 3. The minimum neurological score is 0 and the maximum is 18, which means that behavioral deficits were graded on a total score from 0 to 18. A lower score represents serious neurological deficits. Neurological deficits were assessed by a"blinded" co-worker. All sham-operated animals had a normal score.

2.5. Assay of brain water content

Rats ($n = 6$) were sacrificed after 24 h of SAH. The brain was rapidly divided into the right and left hemispheres, cerebellum, and brainstem and was weighed immediately (wet weight). The brain samples were dried in an oven at 105 °C for 24 h and weighed again (dry weight). The brain water content was calculated as [(wet weight − dry weight)/wet weight] × 100% [37].

2.6. Assay of BBB disruption

Evans blue dye (2%, 5 ml/kg) was administered into the left femoral vein after 24 h of SAH and allowed to circulate for 60 min. Then, the rats ($n = 6$) were sacrificed under deep anesthesia by intracardial perfusion with phosphate-buffered saline (PBS). Subsequently, the brain was removed and divided into 4 parts as described above. Brain samples were weighed and homogenized in 3 ml PBS and then centrifuged at 15,000 × g for 30 min. The supernatant (0.7 ml) was mixed with an equal volume of trichloroacetic acid with ethanol (1:3). After overnight incubation at 4 °C, the samples were centrifuged at 15,000 × g for 30 min, and the supernatant was measured by spectrofluorophotomery at an excitation wavelength of 620 nm and an emission wavelength of 680 nm.

2.7. Histology and TUNEL staining assay

After 24 h of SAH, rats ($n = 2$) were sacrificed and perfused intracardially with PBS (pH 7.4), and 4% paraformaldehyde (pH 7.4). Brains were collected and placed at 4 °C in the same fixative for 2 d. Then, the brains were frozen in tissue-freezing media and cut in 10 μm sections. TUNEL staining was performed according to the manufacturer's protocol (Roche Inc,Basel,Switzerland) and examined under a laser scanning confocal microscope (LSM-710; Zeiss).

2.8. Western blot

Western blot was performed as described previously[3]. Briefly, the right basal cortical sample which faced the blood clot was homogenized and centrifuged at 1000 g for 10 min at 4 °C. The resulting supernatants were further centrifuged, and protein content was measured using the DC Protein Assay (Bio-Rad). An equal amount of protein (60 μg) was resuspended in loading buffer, denatured at 95 °C for 5 min, and loaded onto sodium dodecyl sulfate–polyacrylamide gels. The proteins were electrophoresed at 80 V for 3 h and transferred to polyvinylidene fluoride membranes at 100 V for 2 h. The membranes were blocked with nonfat dry milk buffer and probed overnight at 4 °C with primary antibodies for caspase-3 (1:1000, sc-22140, Santa Cruz Biotechnology), matrix metallopeptidase 9 (MMP-9) (1:800, sc-12759, Santa Cruz Biotechnology) and β-actin (1:2000, Santa Cruz Biotechnology). The membranes were processed with horseradish-peroxidase-conjugated secondary antibodies for 1 h at 21 °C. The protein band densities were detected by X-ray film and quantified by the Image J software (NIH).

2.9. Statistical analysis

Data were presented as the means ± SEM. Statistical significance was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. $P < 0.05$ was considered statistically significant.