

# Role of endothelin-1 and its receptors in cerebral vasospasm following subarachnoid hemorrhage

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**Abstract.** Cerebral vasospasm (CVS) is a severe complication of subarachnoid hemorrhage (SAH), and endothelin-1 (ET-1) may be involved in its pathogenesis. The present study aimed to investigate the expression of ET-1 in cerebrospinal fluid (CSF) in patients with SAH and to analyze rat arterial contractility and the expression levels of ET-1 receptors *in vitro*. CSF samples were collected from 28 patients and the expression levels of ET-1 were measured. Rat cerebral basilar arteries were isolated and incubated with hemorrhagic or clear CSF. Contractility, as well as ET<sub>A</sub> and ET<sub>B</sub> mRNA expression were measured. ET-1 levels in CSF increased and reached a peak within the initial 5 days after SAH onset and then gradually subsided. After 12 or 24 h, the contraction of arteries incubated in hemorrhagic CSF was substantially stronger than those in clear CSF. The mRNA expression levels of endothelin receptor type A and B in arteries incubated in hemorrhagic CSF were significantly higher than those in clear CSF. ET-1 and its receptors may be involved in the pathogenic mechanism of CVS following SAH. ET-1 expression in CSF may be used as a marker in CVS and its receptors may provide novel therapeutic targets in CVS.

## Introduction

Subarachnoid hemorrhage (SAH) refers to bleeding within the subarachnoid space, which is a critical condition with high

morbidity and mortality. The annual incidence of SAH has been estimated to be approximately 9/100,000 in the general population, worldwide (1). Cerebral vasospasm (CVS) is one of the most serious complications of SAH and is an independent risk factor for poor prognosis (2). CVS most commonly occurs within 3 to 15 days from the onset of SAH, and reaches peak severity between 7 and 10 days. CVS is characterized by upregulated vasoconstrictor and downregulated vasodilator expression, as well as vascular smooth muscle cell hypercontractility (3).

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide of endothelial origin and may be associated with the pathogenesis of CVS following aneurysmal SAH (4). ET-1-induced vasoconstriction is mediated by two G-protein-coupled receptors: Endothelin receptor type A (ET<sub>A</sub>) and endothelin receptor type B (ET<sub>B</sub>) (5). In experimental animal models of SAH, it has been demonstrated that cerebral arteries are more sensitive to ET-1 (6,7), and increased levels of ET<sub>A</sub> and ET<sub>B</sub> mRNA have been reported (8,9). Additionally, ET-1 receptor antagonists, particularly antagonists of both ET<sub>A</sub> and ET<sub>B</sub> receptors, prevent and relieve SAH-induced CVS (10,11). Some scholars have speculated that increased levels of ET-1 in the cerebrospinal fluid (CSF) of patients with SAH may induce hypercontractility in cerebral arteries, thereby contributing to the occurrence of CVS (12).

The present study aimed to investigate the expression levels of ET-1 in the CSF of patients with aneurysmal SAH. In addition, arterial contractility and ET-1 receptor expression (ET<sub>A</sub> and ET<sub>B</sub> mRNA) was analyzed *in vitro*. Based on the present study, the therapeutic applications of the antagonist for ET-1 receptors should be assessed in further studies.

## Materials and methods

**Participants and CSF sample collection.** The present study was approved by the Institutional Review Board and Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China; approval no. 2016-074). A total of

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16 patients were enrolled, who were diagnosed with aneurysmal SAH based on computed tomography (CT) and/or digital subtraction angiography (DSA) evidence. The individual clinical severity of SAH was assessed according to the World Federation of Neurological Surgeons scale and the Fisher CT grading system (13,14). Additionally, 12 patients with hydrocephalus were enrolled as controls.

The inclusion criteria were as follows: i) Between the ages of 18 and 80 years old; ii) diagnosed with aneurysmal SAH by CT and/or DSA; iii) admission within 48 h from onset of SAH; iv) Fisher grade III or IV, which is radiologically typical and prone to CVS (14); and v) consent to participate was provided.

CSF samples were collected from patients with aneurysmal SAH and hydrocephalus, which were classified into a SAH group and NPH group, respectively. All CSF samples were obtained via lumbar puncture or lumbar subarachnoid space catheter. The CSF samples were collected within seven-point time frame covering 0-10 days after the onset of SAH. All CSF samples were centrifuged at (4,130 x g) for 15 min at room temperature, following which the supernatant was frozen at -80°C. The hemorrhagic CSF samples can be diluted by fresh-made MOPS buffer into different concentrations, including 25 and 50%.

**Measurement of ET-1 levels.** ET-1 concentrations in CSF samples were measured using an ELISA kit (cat. no. F20030; Shanghai Xitang Biotechnology Co., Ltd., Shanghai, China) at the National Key Laboratory of China Pharmaceutical University.

**Animal selection and artery incubation.** Animal experiments were approved by the Animal Experiment Management Committee of Xi'an Jiaotong University (Xi'an, China), and all measures were taken to minimize animal discomfort. Specific pathogen-free Sprague-Dawley rats (age, 6-months; weight, 300-400 g; 30 males and 30 females) were provided by the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). The rats were maintained in a controlled facility at 25°C and 50% humidity, under a 12 h light/dark cycle with free access to standard chow and water.

The rats were sacrificed by carbon dioxide overdose followed by cervical dislocation. The brain was immediately removed and immersed in fresh-made ice-cold MOPS buffer. Its constituents are shown in Table I. The cerebral basilar arteries were isolated using a stereomicroscope and were subsequently cut into segments of approximately 1 mm in length. The artery segments were put in a culture plate with 2 ml medium (hemorrhagic CSF or clear CSF from SAH and hydrocephalus patients, respectively) containing antibiotics (1x10<sup>5</sup> U/l penicillin and 100 mg/l streptomycin). The culture plate was put into a box with a constant temperature of 37°C, which was continuously ventilated with 5% CO<sub>2</sub> for 6, 12 or 24 h.

**In vitro myographic experiments.** Artery segments were placed in MOPS fluid and threaded by two filaments (diameter, 40 µm) prior to mounting in a small vessel PowerLab myograph (Danish Myo Technology A/S, Aarhus, Denmark). One filament was connected to a tension transducer linked

Table I. Constituents of MOPS buffer and 63.5 mM K<sup>+</sup> MOPS buffer (/L).

Constituents	MOPS buffer	63.5 mM K <sup>+</sup> MOPS buffer
Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	0.4295 g	0.4295 g
MOPS	0.418 g	0.418 g
EDTA	0.006 g	0.006 g
NaCl	8.15 g	4.945 g
KCl	0.35 g	4.475 g
CaCl <sub>2</sub>	0.1775 g	0.1775 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.295 g	0.295 g
Glucose	1 g	1 g

All constituents were dissolved in distilled water and adjusted to pH=7.4 using NaOH or HCL to 11 total volume.

to the PowerLab system, and the other was connected to the fine-tuning device that adjusts the distance between the two filaments. Then, the installed vascular rings were laid in a bath chamber containing 5 ml MOPS fluid with continuous ventilation and a pH of 7.4. All of the vessels were allowed to equilibrate for 30 min at room temperature. When the baseline was stable, the initial vascular tension was changed to 1.5 mN. Following this, 5 ml fresh-made potassium rich (63.5 mM K<sup>+</sup>) MOPS fluid was added into the bath chamber twice to evaluate contractility. The constituents of (63.5 mM K<sup>+</sup>) MOPS buffer are also shown in Table I. Between the twice potassium rich MOPS buffer, the vessel segments were washed using common MOPS buffer three times. Vessels with a contraction difference of <10% were included for further analyses. Finally, ET-1 (ALX-155-001-PC05, Enzo Life Sciences, Inc., Farmingdale, NY, USA) and sarafotoxins 6c (S6c, ET-1 analogue; S6545, Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) were added into the bath chamber at gradually increasing concentrations to obtain the concentration response curves. The concentration of S6c ranged from 10<sup>-11</sup> to 10<sup>-7.5</sup> mol/l, while that of ET-1 ranged from 10<sup>-11</sup> to 10<sup>-7</sup> mol/l. The contractile response of the vessels was expressed as a percentage of the contraction induced by potassium rich MOPS fluid. The maximal contraction was indicated by E<sub>max</sub>, and pEC<sub>50</sub> denoted the negative logarithm of the concentration that elicits half of the maximal response.

**Detection of ET<sub>A</sub> and ET<sub>B</sub> mRNA expression.** Total cellular RNA was extracted from rat cerebral arteries, including anterior, middle and posterior cerebral artery as well as basilar artery that had been incubated at 37°C for 24 h. Reverse transcription of total RNA to single-stranded cDNA was performed using SuperScript™ IV Reverse Transcriptase (18090010, Thermo Fisher Scientific, Inc., Waltham, MA USA) in a 12 µl reaction system. RNA was incubated at 65°C for 5 min for denaturation, following which it was immediately cooled on ice. The reverse transcription master mix was added to the tube, and the reaction was performed at 42°C for 1 h and 70°C for 5 min. Polymerase chain reaction (PCR) was performed using a SYBR PreMix Ex Taq™ kit

Table II. Primer sequences used for polymerase chain reaction.

Gene	Forward primer sequences (5'-3')	Reverse primer sequences (3'-5')
ET <sub>A</sub>	GTCGAGAGGTGGCAAAGACC	ACAGGGCGAAGATGACAACC
ET <sub>B</sub>	GATACGACAACCTCCGCTCCA	GTCCACGATGAGGACAATGAG
EF-1 <sub>α</sub>	CCCCTCCAGGACGTTTACAAA	CACACGGCCCACAGGTACA

ET<sub>A</sub>, endothelin receptor type A; ET<sub>B</sub>, endothelin receptor type B.

Table III. Demographic and clinical data of enrolled patients.

Characteristics	SAH group	NPH group
Gender (male/female)	8/8	6/6
Age (years)	52.38±13.24	30.75±3.234
Fisher grade III (n)	11	-
Fisher grade IV (n)	5	-
Hunt-Hess grade II-III (n)	12	-
Hunt-Hess grade IV-V (n)	4	-

(Takara Bio, Inc., Otsu, Japan) in an iCycler iQ5 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). This system automatically monitors the binding of fluorescent SYBR Green dye to double-stranded DNA during each PCR amplification cycle. PCR was performed in a 10 μl reaction system. The PCR amplification procedure started at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec, 60°C for 60 sec and 72°C for 15 sec, and ended at 55°C for 30 sec. Dissociation curves were identified for the PCR products, and the 2<sup>-ΔΔC<sub>q</sub></sup> method was used to analyze the mRNA levels (15). The primer sequences used for RT-PCR were listed in Table II.

**Statistical analysis.** SPSS 19.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. Continuous data were presented as the mean ± standard error. The comparison between groups was performed using a one-way analysis of variance or Kruskal-Wallis test, based on the homogeneity of variance. Multiple comparisons among groups were performed using the Student-Neuman-Keuls method. P≤0.05 was considered to indicate a statistically significant difference.

## Results

**Alterations in CSF ET-1 expression levels.** The clinical data of enrolled patients were summarized in Table III. The ET-1 levels in the CSF samples that were collected within seven-time frames following the onset of SAH are presented in Table IV. The ET-1 levels in CSF appeared to increase in the initial five days, reaching a peak within 3 to 5 days after onset of SAH, but then gradually subsided. However, comparison between the seven groups showed no significant difference (P>0.05).

**Contraction of arteries incubated with hemorrhagic CSF of different concentrations.** In order to determine the best

Table IV. ET-1 expression levels in the hemorrhagic CSF samples.

Sampling time (T; days)	ET-1 (pg/ml)
T≤1.5	93.78±13.10
1.5<T≤2.5	105.01±17.28
2.5<T≤3	102.74±9.58
3<T≤5	107.54±18.98
5<T≤7	102.18±12.58
7<T≤10	104.02±17.42
T>10	91.75±4.14

ET-1, endothelin-1; CSF, cerebrospinal fluid.

functional concentration, the hemorrhagic CSF was diluted in MOPS buffer and classified into three different concentrations (25, 50 or 100%). Rat basilar arteries were incubated with CSF of different concentrations for 12 h, and *in vitro* contraction responses to ET-1 or S6c were observed. Statistical analysis demonstrated a positive association between the concentration and contraction degree, with a significant difference (P<0.05). Based on this result, 100% hemorrhagic CSF to was used at the culture media (data not shown).

### Contraction of arteries for different incubation durations.

The E<sub>max</sub> and pEC<sub>50</sub> of unin. cubated fresh arteries and those of arteries incubated with clear CSF or hemorrhagic CSF for 6, 12 or 24 h are presented in Table V. The contraction curves are presented in Figs. 1 and 2. In unincubated fresh arteries, ET-1 induced significant vasoconstriction, while S6c resulted in no vasoconstrictive effects.

After a 6 h incubation, all of the artery rings contracted, and those cultivated in clear CSF exhibited a more marked contraction. Statistical analysis demonstrated significant differences in the context of S6c concentrations ranging from 10<sup>-9.5</sup> to 10<sup>-7.5</sup> mol/l, or ET-1 concentrations ranging from 10<sup>-10</sup> to 10<sup>-8.5</sup> mol/l (Fig. 1A).

After a 12 h incubation, all of the artery rings contracted, and those cultivated in hemorrhagic CSF demonstrated a larger contraction. Statistical analysis revealed significant differences in the context of S6c concentrations ranging from 10<sup>-9.5</sup> to 10<sup>-8.5</sup> mol/l, or ET-1 concentrations ranging from 10<sup>-9.5</sup> to 10<sup>-7.5</sup> mol/l (Fig. 1B).

After a 24 h incubation, all of the artery rings contracted, and those cultivated in hemorrhagic CSF exhibited a more

Table V.  $E_{\max}$  and  $pEC_{50}$  of artery rings.

Incubation duration	Incubation medium	S6c			ET-1		
		n	$E_{\max}$ (% $K^+$ )	$pEC_{50}$	n	$E_{\max}$ (% $K^+$ )	$pEC_{50}$
Control	Not incubated	7	0±0.00	0±0.00	7	156.1±8.71	8.9±0.12
6 h	SAH	7	51.43±19.64	9.6±0.24	7	156.1±15.81	8.5±0.05
	NPH	7	122.0±15.85	9.2±0.07	7	170.0±31.85	9.3±0.07
12 h	SAH	7	99.00±28.89	9.7±0.14	7	153.0±16.53	9.4±0.08
	NPH	7	77.14±17.73	9.5±0.01	7	137.7±8.26	9.5±0.06
24 h	SAH	7	199.3±10.70	9.6±6.82	7	242.1±33.13	9.7±7.04
	NPH	7	132.3±7.00	9.7±0.18	7	132.0±13.78	9.7±0.11

n indicates the number of samples in each group. NPH, clear cerebrospinal fluid; SAH, subarachnoid hemorrhagic cerebrospinal fluid; ET-1, endothelin-1; S6c, sarafotoxins 6c.

marked contraction. Statistical analysis demonstrated significant differences in the context of S6c concentrations ranging from  $10^{-9}$  to  $10^{-7.5}$  mol/l, or ET-1 concentrations ranging from  $10^{-9}$  to  $10^{-7}$  mol/l (Fig. 1C).

**Contraction response to S6c or ET-1.** In arteries incubated with hemorrhagic CSF, S6c and ET-1 both induced vasoconstriction. Following the administration of S6c, the contraction degree was positively associated with the incubation period. When compared to fresh arteries, statistical analysis revealed significant differences in the context of S6c concentrations, ranging from  $10^{-9.5}$  to  $10^{-7.5}$  mol/l; there was also a significant difference in contraction degree between arteries incubated for 12 or 24 h (Fig. 2). Following the administration of ET-1, the contraction of arteries incubated for 6 h were not significantly different compared with the fresh arteries, and the arteries incubated for 24 h demonstrated the most remarkable contraction; statistical analysis revealed a significant difference in contraction degree between 24-h incubated arteries and fresh arteries in the context of a ET-1 concentration ranging from  $10^{-9.5}$  to  $10^{-7}$  mol/l.

**Expression of  $ET_A$  and  $ET_B$  mRNA.** The detected expression levels of  $ET_A$  and  $ET_B$  mRNA are summarized in Table VI and Fig. 3. The expression levels of  $ET_A$  mRNA in the incubation groups were significantly higher compared with the control group ( $P < 0.05$ ). The expressions level of  $ET_B$  mRNA in the hemorrhagic CSF-incubation group was significantly higher compared with the control group ( $P < 0.01$ ), while there was no significant difference in the expression level of  $ET_B$  mRNA between the clear CSF-incubated arteries and the control arteries ( $P > 0.05$ ). There was no significant difference in the expression levels of  $ET_A$  or  $ET_B$  mRNA between the hemorrhagic CSF-incubated arteries and the clear CSF-incubated arteries ( $P > 0.05$ ).

## Discussion

CVS is a serious complication of SAH, which may lead to fatal outcomes. The pathogenetic mechanisms remain poorly understood (16). Previous studies have demonstrated

how vasospasm may develop following SAH, which involves altered expression levels of various vasoactive substances and specific receptors, as well as the matrix metalloproteinase family, inflammatory pathways, altered metal ion concentrations in CSF and high cholesterol levels (17-19). Among the mechanisms noted above, an increased level of contractile vasoactive substances and unregulated expression of their receptors are the most immediate causes for CVS following SAH. As a well-established, potent and long-lasting vasoconstrictor, ET-1 serves a crucial role in regulating the vascular tension (20). Previous studies have demonstrated that ET-1 levels in both plasma and CSF may increase in patients with SAH (21), and ET-1 causes dose-dependent vasoconstriction in animal models (7). The present study also drew similar conclusions, with the results indicating that ET-1 levels in the CSF of patients with SAH increased, and arteries incubated with hemorrhagic CSF from patients with SAH constricted in a dose-dependent manner in response to ET-1 and S6c. The present study used hemorrhagic CSF from patients with SAH to incubate rat basilar arteries, as this can better mimic the reaction of cerebral vessels of patients following SAH onset (22). However, the definitive pathogenic effects of ET-1 in CVS remain unclear. In the current study, the dynamic alterations in ET-1 expression in the CSF of SAH patients were demonstrated, and the contractility and expression levels of endothelin receptors in rat cerebral arteries incubated in various conditions were investigated.

Suzuki *et al* (23) revealed that the ET-1 expression levels in both the plasma and CSF of patients with SAH classified as Fisher grade III to IV were significantly higher, compared with those in patients with SAH classified as Fisher grade I or II. The present study only included patients with SAH classified as Fisher grade III or IV, who were more prone to develop CVS; CSF samples were collected within seven-time frames covering 0-10 days after SAH onset to observe the dynamic alterations in ET-1 levels. The findings of the current study indicated that the ET-1 expression levels in CSF increased in the initial 5 days, reaching a peak within 3 to 5 days following SAH onset, and then gradually subsided. It was speculated that these alterations may be due to a sequential process including blood flowing into the

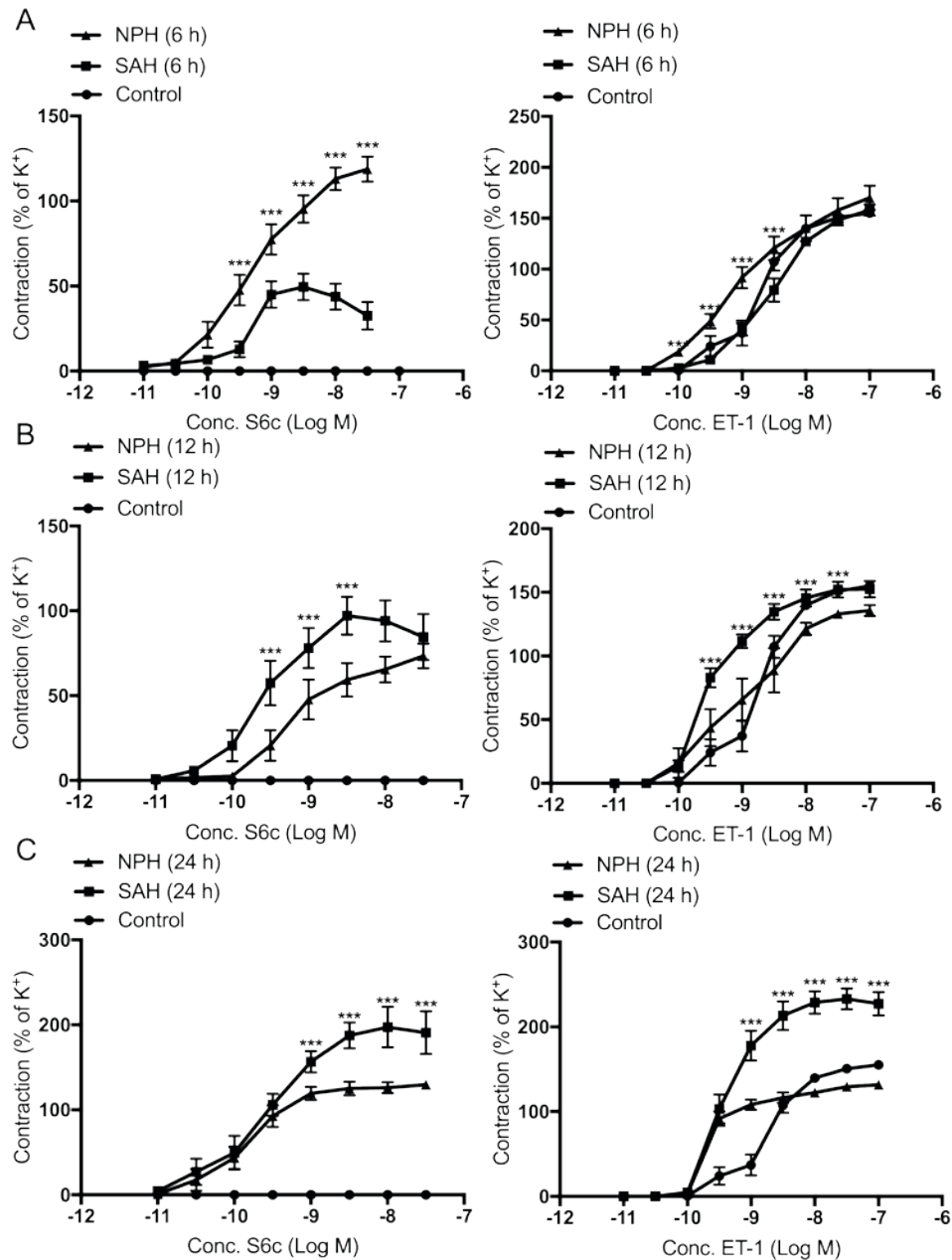


Figure 1. Contraction curves following the administration of S6c or ET-1 for (A) 6, (B) 12 or (C) 24 h. The control groups were fresh arteries and not incubated. NPH or SAH group means arteries were incubated with clear CSF or hemorrhagic CSF, and control group means arteries were not incubated. For each group, n=7. \*\*\*P<0.05 vs. control group. CSF, cerebrospinal fluid; NPH, clear CSF; SAH, subarachnoid hemorrhagic CSF; Conc., concentration; ET-1, endothelin-1; S6c, sarafotoxins 6c.

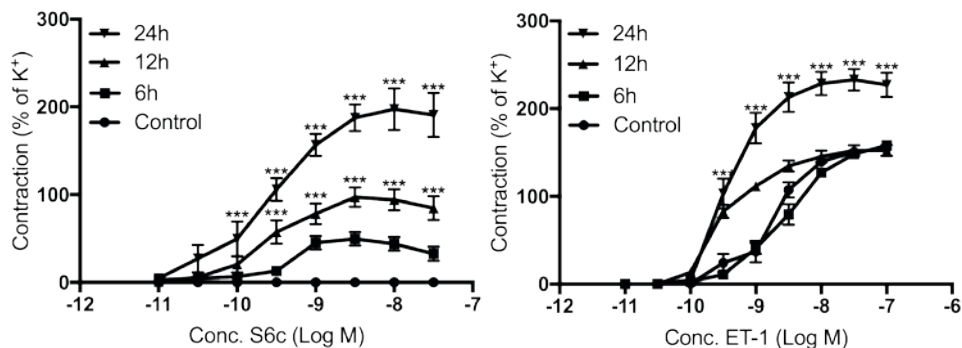


Figure 2. Contraction curves of vessels in the SAH group incubated for 6, 12 or 24 h following the administration of S6c or ET-1. The control groups were fresh arteries and not incubated. For each group, n=7. \*\*\*P<0.05 vs. control group. Conc., concentration; ET-1, endothelin-1; S6c, sarafotoxins 6c.

Table VI. Expression levels of ET<sub>A</sub> and ET<sub>B</sub> mRNA.

Groups	n	ET <sub>B</sub> mRNA	n	ET <sub>A</sub> mRNA
		Relative expression level		Relative expression level
Control	5	0.0229±0.0041	5	0.0136±0.0038
NPH	4	0.0808±0.0242	5	0.2375±0.0875
SAH	5	0.1531±0.0235	5	0.2606±0.0644

n indicates the number of samples in each group. The control groups were fresh arteries and not incubated. NPH, clear cerebrospinal fluid; SAH, subarachnoid hemorrhagic cerebrospinal fluid; ET<sub>A</sub>, endothelin receptor type A; ET<sub>B</sub>, endothelin receptor type B.

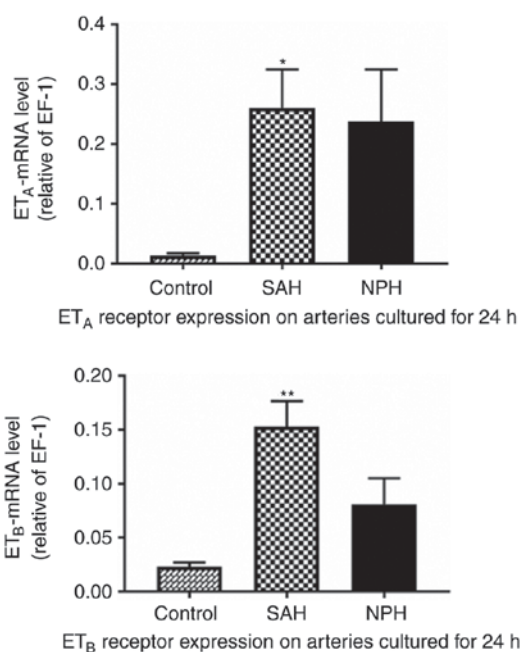


Figure 3. Relative expression levels of ET<sub>A</sub> and ET<sub>B</sub> mRNA. The control groups were fresh arteries and not incubated. The reference gene was elongation factor 1 alpha (EF1- $\alpha$ ). For each group, n=5. \*P<0.05, \*\*P<0.01 vs. control group. CSF, cerebrospinal fluid; NPH, clear CSF; SAH, subarachnoid hemorrhagic CSF.

subarachnoid space, erythrocyte splitting, and their splitting products, such as oxyhemoglobin and oxygenase-1. These can stimulate cerebral vessels both physically and chemically (24,25). Furthermore, previous studies demonstrated that patients with SAH may be at a high risk of developing CVS within 4-10 days following aneurysm rupture (3). This clinical phenomenon is consistent with the results of the present study, in that the time period of the higher CVS risk was similar to the higher levels of ET-1 detected in the CSF of SAH patients. The peak expression of ET-1 in the CSF of SAH patients appeared within 3-5 days and remained at a high level until 10 days after SAH onset. Therefore, it was speculated that ET-1 expression in the CSF may be a potential biomarker to predict CVS following SAH. However, statistical analysis revealed no significant difference, which may be due to the limited sample size; therefore, the exact

role of ET-1 expression in patients with SAH requires further research.

To evaluate the expression of endothelin receptors and the arterial contractility, an *in vitro* experiment was performed using artery samples isolated from rat models. The arterial rings were incubated with hemorrhagic CSF or clear CSF, and ET-1 and S6c were administered to induce arterial contraction. It was demonstrated that S6c induced a significant contraction in CSF-incubated arteries but not in fresh arteries, whereas ET-1 induced contraction in both CSF-incubated and fresh arteries. A potential explanation for this phenomenon is that S6c is a selective ET<sub>B</sub> receptor agonist, and ET<sub>B</sub> receptors are infrequently expressed in fresh vessels. However, they may greatly increase following CSF incubation. Nevertheless, ET-1 induces arterial contraction by both ET<sub>A</sub> receptors and ET<sub>B</sub> receptors, and ET<sub>A</sub> receptors are widely expressed in both fresh and CSF-incubated arteries (26). In the present study, as ET-1 or S6c concentrations increased, the arterial contraction increased. In arteries incubated in clear CSF, an incubation period of 6 h was associated with the most obvious arterial contraction, whereas in arteries incubated in hemorrhagic CSF, an incubation period of 12 or 24 h was associated with the strongest arterial contraction, and the contraction degree was associated with the incubation duration and CSF concentration. This discrepancy may be because there were not only vasoconstrictors but also a small quantity of short-acting vasodilators in hemorrhagic CSF (27,28).

Furthermore, significantly elevated expression levels of both ET<sub>A</sub> and ET<sub>B</sub> mRNA in arteries incubated with hemorrhagic CSF were detected, compared with the expression levels in fresh arteries. However, there was no significant difference in the expression levels of ET<sub>A</sub> or ET<sub>B</sub> mRNA between the hemorrhagic CSF-incubated arteries and the clear CSF-incubated arteries. Zuccarello *et al* (29) proposed that ET<sub>A</sub> receptors induce vasoconstriction, while ET<sub>B</sub> receptors mediate vasodilation in physiological conditions. Certain reports have also speculated that SAH may result in an altered endothelial-dependent action of ET-1 and may lead to the upregulation of ET<sub>B</sub> receptors in vascular smooth muscle cells, thereby contributing to vasoconstriction (30,31). It was therefore considered that the enhanced contractility of hemorrhagic CSF-incubated arteries in the present study may have been more associated with upregulated expression of ET<sub>B</sub> receptors, rather than ET<sub>A</sub> receptors. In previous studies, scholars reported that clazosentan, a nonpeptide selective ET<sub>A</sub> receptor antagonist, significantly reduces the occurrence of CVS following SAH, but that it did not improve clinical outcomes (32-34). This may be because this selective ET<sub>A</sub> receptor antagonist cannot antagonize ET<sub>B</sub> receptors, which may serve a more important role in the pathogenesis of CVS following SAH (32). According to the result of the present study and the unsatisfactory treatment effect of clazosentan, the therapeutic efficacy of ET receptor antagonists acting on both ET<sub>A</sub> receptor and ET<sub>B</sub> receptor requires further study.

The role of ET-1 and its receptors in the pathogenetic mechanism of CVS remains unclear. Previous studies have indicated that the mitogen-activated protein kinase (MAPK) signaling pathway may be a promising research avenue, as the activation of MAPKs may increase the expression of endothelin

receptors (35). However, the role of MAPK phosphatases, the endogenous MAPK inhibitors, has not been investigated in CVS. Additionally, the elevated expression of ET<sub>A</sub> receptors was reported to be associated with the activation of extracellular regulated protein kinase in vascular endothelial cells, as well as intracellular protein kinase C, protein kinase A and phosphatidylinositol 3-kinase (36). The causative role of ET-1 and its receptors in CVS warrants further research.

The sample size in the current study was limited, and in the future studies the cohort size of patients with SAH should be expanded to obtain more CSF samples. Additionally, hemodynamic parameters will be recorded using CT perfusion techniques, including cerebral blood flow, cerebral blood volume, and mean transit time, to further investigate the effects of ET-1 on cerebral vessels, particularly microvessels.

In conclusion, ET-1 and its receptors may be involved in the pathogenic mechanism of CVS following SAH. It was demonstrated that the dynamic alterations in ET-1 expression levels in CSF were parallel with the clinical development of CVS in patients with SAH, indicating that ET-1 may be a helpful biomarker for the prediction and diagnosis of CVS. The expression levels of ET<sub>A</sub> and ET<sub>B</sub> mRNA were upregulated in CSF following SAH, which may be associated with the hypercontractility of cerebral arteries. ET-1 receptors may provide novel therapeutic targets in CVS.

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#### Availability of data and materials

All data generated and analyzed during the present study is included in this published article.

#### Authors' contributions

GL, YXC and CX conceived and designed the experiments. YWC, WL and XD performed the experiments. YWC, WL and RJ analyzed the data. HY and XL helped testing the levels of ET-1 in the CSF samples. JL designed the concentration gradient of the drugs. YWC wrote the manuscript. All authors have read and approved this manuscript.

#### Ethics approval and consent to participate

The present study was approved by the Institutional Review Board and Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China; approval no. 2016-074). Animal experiments were approved by the Animal Experiment Management Committee of Xi'an Jiaotong University (Xi'an, China), and all measures were taken to minimize animal discomfort.

#### Patient consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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