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Research Report

# Rapidly increased vasopressin promotes acute platelet aggregation and early brain injury after experimental subarachnoid hemorrhage in a rat model

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ABSTRACT

*Objective:* To investigate the dynamic expression of vasopressin and its potential role in rat brain tissue after experimental subarachnoid hemorrhage (SAH).

*Methods:* Male Sprague-Dawley rats were divided into 10 min, 1 h, 6 h, 24 h, 48 h and 72 h groups. The SAH model was established by endovascular puncture. ELISA and immunohistochemistry were performed to evaluate dynamic expression of vasopressin. Immunohistochemistry of GPIIb/IIIa integrin was used to assess platelet aggregation. Double immunofluorescence labeling was carried out to observe the reaction between vasopressin and platelet. Early brain injury was evaluated by apoptotic cells counting. Neurobehavioral score was performed to assess neuroprotective role of SR 49059 (a selective antagonists of vasopressin receptor).

*Results:* In peripheral blood and hypothalamus, vasopressin increased rapidly at 6 h and 24 h. Expression of GPIIb/IIIa integrin peaked at 24 h in cortex and hippocampus. Immunofluorescence showed that vasopressin and GPIIb/IIIa integrin located at the same site. Administration of SR 49059 significantly decreased platelet aggregation and number of apoptotic cells. The neurobehavioral score was promoted significantly after the intervention.

*Conclusion:* The results indicate that rapidly increased vasopressin could induce platelet aggregation and contribute to early brain injury after SAH.



## 1. Introduction

Aneurysmal subarachnoid hemorrhage (SAH) is a serve cerebrovascular disease. The incidence of aneurysmal SAH is 6–7 per 100,000 persons per annum, and the fatality rate in acute phase is about 50% (van Gijn et al., 2007). Meanwhile, most of survivors suffer from delayed ischemic neurological deficit (DIND), which leads to a low quality of life. In the last decade,

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the occurrence of DIND was mostly ascribed to delayed cerebral vasospasm. However, evidence was showed that Clazosentan, an ET-1A antagonist which could reduce the incidence of vasospasm, fails to improve neurological outcomes (Macdonald et al., 2011), indicating delayed cerebral vasospasm might not be the main reason of DIND (Sehba et al., 2012).

Early brain injury is a group of pathophysiological processes that occur during the first 72 h after SAH and also contributes to DIND (Munoz-Guillen et al., 2013; Sehba et al., 2012). Recent studies noted microthrombosis as one of the possible mechanisms leading to early brain injury (Munoz-Guillen et al., 2013). Both clinical and experimental researches have demonstrated the formation of microthrombosis early after SAH and its impact on cerebral blood flow (CBF) (Akopov et al., 1996; Friedrich et al., 2012; Friedrich et al., 2010). In biopsy of SAH patients, thrombosis was found in microvessels of brains 2 days after SAH took place (Stein et al., 2006). In rats, platelet aggregation was detected in microvessels 10 min after SAH model was established (Friedrich et al., 2010; Sehba et al., 2005).

Platelet aggregation is a vital step of microthrombosis formation. However, the mechanism of platelet aggregation after SAH is not completely understood. Arginine-vasopressin was reported to play a pivotal role in thrombosis (Siess et al., 1986). Thomas et al. (1983) demonstrated the activation of V1a receptor, one of vasopressin receptors located on platelets, led to release of arachidonic acid and production of arachidonate metabolites, which further activated platelets and promoted platelet aggregation. Moreover, evidence has been shown that vasopressin went through a rapid and significant increase after SAH in both rats and patients (Kleindienst et al., 2004; Yuan et al., 2010). Therefore, we postulate that increased vasopressin might induce excessive platelet aggregation during early phase of SAH through its interaction with V1a receptor. Enhanced platelet aggregation may contribute to the formation of microthrombosis and finally lead to early brain injury. In this article, we first studied the spatiotemporal expression of vasopressin and platelet aggregation during acute phase in a rat SAH model. We used GPIIb/IIIa integrin (a glycoprotein located on the membrane of platelet) antibody to detect platelet aggregation (Sehba et al., 2005). We also used immunostaining to assess spatial relationship between vasopressin and platelets. In the second part, we applied V1a antagonist to investigate the correlation between vasopressin and platelet aggregation. We hope this research may provide a new understanding of mechanisms regarding in early brain injury, or develop new therapeutic target for SAH.

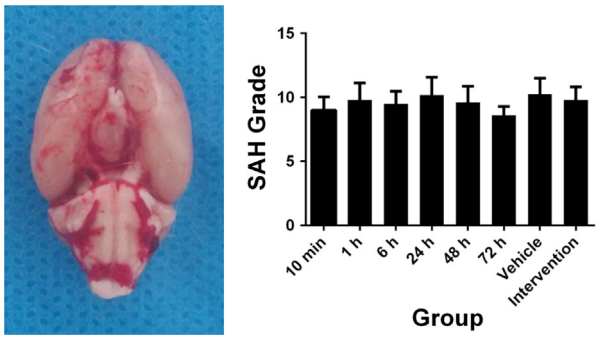


**Fig. 1 – SAH grade and gross observation of brain tissue in 24 h group. Brains included into current study suffered from moderate SAH (scores range from 8 to 12). In 24 h group, there are apparent blood clots on the surface of rat brain.**

## 2. Results

### 2.1. *Mortality and SAH grading*

The mortality in this study is 0% in normal group, 0% in sham group and 18.04% in SAH related groups. Brains were photographed immediately after removal. Twenty-four hours group had big blood clots (Fig. 1). Normal and sham group exhibited no subarachnoid hemorrhage or any clot formation. Although blood clots in 72 h group were a bit faded, scores of all included SAH brains ranged from 8 to 12. This meant that rats suffer from moderate SAH (Fig. 1).

### 2.2. *Expression of secretive vasopressin determined by ELISA*

Vasopressin values of each group were calculated based on the standard curve (Fig. 2). Sham and normal group showed a stable curve. In SAH groups, vasopressin increased rapidly after operation, peaked at 6 h point and then gradually declined. The secretion of vasopressin was significantly increased in each SAH subgroup compared to its time-matched sham group ($P<0.01$). Values were significant higher at 6 h and 24 h than those of other SAH subgroups ($P<0.01$).

### 2.3. *Expression of vasopressin determined by immunohistochemistry*

We chose hypothalamus to observe the expression of vasopressin (Fig. 3). On sections, there were no substantial differences between sides ipsi- and contralateral to the hemorrhage. In normal and sham groups, level of vasopressin was in constantly low. Statistics showed no significant difference

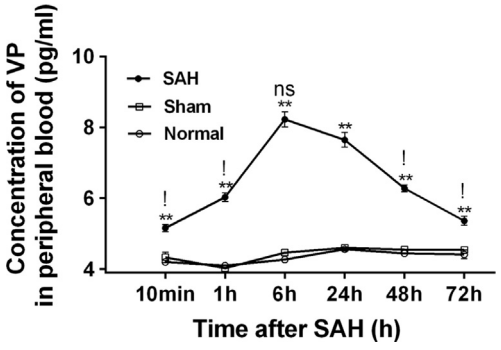


**Fig. 2 – ELISA of vasopressin in peripheral blood. The data represent dynamic concentration of vasopressin in normal, sham and SAH groups. Data are represented as mean ± SD for five animals per time interval in SAH group. The number of rats in normal or sham group at each time point is three. $^{**}p<0.01$ versus time-matched sham group;! $p<0.01$, ns $p>0.05$ versus the 24 h SAH group.**