SP100B Expression Indexed Hemorrhage in Mouse Models of Cerebral Hemorrhage

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Abstract The aim of the present study was to investigate the expression of SP100B in mouse models with acute cerebral hemorrhage. Mouse models of cerebral hemorrhage were induced using injection of collagenase into the brain. The serum levels of SP100B were detected by ELISA. The expression of SP100B in the hippocampus and other brain tissue were detected by indirect immunofluorescence technique. The mean concentration of serum SP100B was significantly higher in hemorrhage group (0.85 µg/l) than in control group (0.20 µg/l) ($P = 0.0017$). More importantly, the mean value of serum SP100B in both moderate hemorrhage (0.96 µg/l) and severe hemorrhage (1.21 µg/l) had significantly higher compared to hyporrhea group (0.39 µg/l) ($P = 0.0041$ and $P = 0.0009$, respectively). The expression of SP100B also increased in the hippocampus with severe hemorrhage. Additionally, the expression of SP100B was high in the early stage of hemorrhage. SP100B expression was positively related to the severity of hemorrhage in mouse models of cerebral hemorrhage. Serum SP100B might be a noninvasive biomarker for cerebral hemorrhage.

Keywords SP100B · Acute · Cerebral hemorrhage · Biomarker

Introduction

The clinical manifestation of craniocerebral injury is varied in different persons. So, early diagnosis of craniocerebral injury is often challenging. Previous studies reported that SP100B was related to brain damage [1–3]. And the serum levels of SP100B not only reflect the grade of brain injury but also predict the occurrence of cerebral vasospasm. But SP100B can't intuitively display the specific organizational performance of cerebral hemorrhage. In recent years, some progress has been made in the experimental research on cerebral hemorrhage due to the animal model of cerebral hemorrhage [4]. In this study, the expression of SP100B in mouse models with acute cerebral hemorrhage was detected and prognosis of craniocerebral injury was also evaluated.

Materials and Methods

Animals Total of 64 male C57BL mice, 10-week-old, weights ranged from 20 to 25 g, were purchased from Silaike laboratory animal co., Ltd (Shanghai). Throughout the studies, the protocol for these experiments was approved by the special committee on Animal Welfare of Yaan People's Hospital. Mice were randomly divided into four groups, control group, hemorrhage group (including hyporrhea group, moderate hemorrhage group and severe hemorrhage group).

Induction of mouse models with acute cerebral hemorrhage Mouse models of acute cerebral hemorrhage were induced using injection of collagenase into the brain. 0.5 U bacterial collagenase type (VII) of saline solution was injected into the caudate nucleus of C57BL mice by micro-injection pump under the help of stereotaxie apparatus.

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And the same volume of sterile saline only was injected into the caudate nucleus of C57BL mice in the control group.

Measurement of SP100B The blood of mice was collected during early postoperative (within 6 h) and the serum was then prepared for measurement. The serum levels of SP100B were evaluated with ELISA kits (SP100B ELISA Test kit, Biokey™ Company, Shanghai, China) according to the manufacturer's protocol.

Histological examination The expression of SP100B in the brain tissues were detected by indirect immunofluorescence technique.

Statistical analysis All data were analyzed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean $\pm$ SD and analyzed by *t* test. For all tests, A *P* value <0.05 was considered to be statistically significant difference.

Results

Animal models of cerebral hemorrhage were successfully constructed In order to see whether the model is successful constructed, the behavior of mice was assessed. After injection of collagenase, animals rotated on the same side (Fig. 1a), and the rotation became serious on the 5th day after injection. And the walking distance of ipsilateral forelimb was also significantly farther than that of contralateral per minute (Fig. 1b).

Serum levels of SP100B were positively related to the severity of cerebral hemorrhage Early postoperative of cerebral hemorrhage (within 6 h), the mean concentration of serum SP100B was significantly higher in hemorrhage group (0.85 $\mu\text{g/l}$) than in control group (0.20 $\mu\text{g/l}$) ($P = 0.0017$) as shown in Fig. 2a. Our results also showed that the mean value of serum SP100B in both moderate hemorrhage (0.96 $\mu\text{g/l}$) and severe hemorrhage (1.21 $\mu\text{g/l}$) had significantly higher compared to hyporrhea group (0.39 $\mu\text{g/l}$) ($P = 0.0041$ and $P = 0.0009$, respectively) as

Fig. 1 Mouse models of cerebral hemorrhage were induced using injection of collagenase. **a** The direction of rotation (*left*) in mice, contrast (*right*). **b** Walking distance of ipsilateral forelimb was significantly farther than the contralateral (*left*) and control (*right*)

shown in Fig. 2b. So, our study revealed that the expression of SP100B concentration was closely related to the severity of cerebral hemorrhage, though we did not find any significant differences for SP100B levels when comparing severe hemorrhage group and moderate hemorrhage group.

The expression of SP100B was increased in the hippocampus with severe hemorrhage Our study found that the expression of SP100B in hippocampus was also positively related to the severity of hemorrhage as showed in Fig. 3.

SP100B was highly expressed in the early stage of hemorrhage Our study showed that the expression of SP100B was high in the early stage of bleeding (within 6 h) (Fig. 4a) and was reduced in the late stage (3 days) (Fig. 4b). Our study also found that SP100B was fasciculate in the purkinje cells in the early stage of injury (Fig. 4c) and was not expressed in the late stage (Fig. 4d)

Discussion

In this study, we firstly illustrated the relationship between SP100B and cerebral hemorrhage in mouse models. Our study found that SP100B was highly expressed in the serum and hippocampus with severe hemorrhage. So, serum SP100B might be a noninvasive biomarker for cerebral hemorrhage.

SP100B is very important for regulating cell, controlling energy metabolism and conducting cell signaling. SP100B is high expressed in the Glial cell or Schwann cell which belong to central nervous system or peripheral nervous system. After brain injury, SP100B will be released from broken Glial cells and flow into cerebrospinal fluid (CSF), while clinical symptoms and other test index are normal. Therefore, the serum concentration of SP100B will be increased and may be a biomarker for brain injury, when SP100B enter into blood through blood–brain barrier. Researches had found that the concentration of SP100B was relative to the scope and severity of brain and blood–brain barrier damage [3, 5–8].

Hemorrhagic cerebrovascular disease can lead to brain tissue injury, Glial cell edema, degeneration and necrosis and blood–brain barrier damage. So the CSF and blood levels of SP100B increased in patients with Hemorrhagic cerebrovascular disease [9, 10]. Moreover, the serum levels of SP100B were positively related to the severity of neurological symptoms. That is, SP100B may be a biomarker for nervous system injury [9, 11].

This study showed that serum concentration of SP100B was obviously higher in brain hemorrhage groups than in control group. Moreover, the level of serum SP100B was closely related to the severity of cerebral hemorrhage. The

Fig. 2 Serum concentration of SP100B **Fig. 3** SP100B expression was increased in the hippocampus with severe hemorrhage (original magnification: $\times 200$), **a** hyporrhea group; **b** severe cerebral hemorrhage (present patches of fluorescent) **Fig. 4** Expression of SP100B in the early and late stage of hemorrhage (original magnification: $\times 200$), **a** SP100B was highly expressed in the early stage of hemorrhage (within 6 h); **b** SP100B was diffused in the late stage of hemorrhage (3 days); **c** SP100B was fasciculate in purkine cells in the early stage of hemorrhage; **d** SP100B was not expressed in purkine cells in the late stage of hemorrhage

expression of SP100B also increased in the hippocampus with severe hemorrhage. As a result, we hypothesised that serum SP100B was increased with the increase of blood loss. This conclusion was confirmed by indirect immunofluorescence which showed that the expression of SP100B was high in the early stage of hemorrhage.

In conclusion, SP100B expression was positively related to the severity of hemorrhage in mouse models of cerebral hemorrhage. Serum SP100B might be a noninvasive biomarker for cerebral hemorrhage.

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Compliance with Ethical Standards

Conflict of interest We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, this manuscript entitled.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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