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BRAIN RESEARCH

Research Report

Perivascular and perineural extension of formed and soluble blood elements in an intracerebral hemorrhage rat model

GuoLin He[b], TianMing Lü[a,*], BingXun Lu[a], Duan Xiao[a], Jia Yin[a], XiaoJia Liu[a], Guang Qiu[a], Min Fang[a], YuanYuan Wang[a]

[a]Department of Neurology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, PR China
[b]Department of Neurology, Zhanjiang Central Hospital, Zhanjiang 524255, PR China

ARTICLE INFO

Article history:
Accepted 26 February 2012
Available online 4 March 2012

Keywords:
Intracerebral hemorrhage
Animal model
Brain edema
Neuropathology
Neurochemistry

ABSTRACT

The perivascular and perineural extension of hematoma has recently been observed in the brain after intracerebral hemorrhage (ICH), which is formed by the leakage of hematoma via the Virchow–Robin spaces (VRS) and the spaces around the nerve fibers (perineurium). The present study investigated the perivascular and perineural extension of a hematoma by studying the distribution of the formed and soluble blood elements labeled with different fluorescein dyes at different times after ICH in a rat model. The ICH rat model was prepared using a modified double injection method. Autologous blood, which contained fluorescein isothiocyanate (FITC)-labeled RBCs or carboxytetramethylrhodamine (TAMRA)-labeled BSA, was injected into the center of the left caudate nucleus. Brain sections were prepared and observed by overlaying fluorescence and hematoxylin and eosin stained images. The formed blood elements extended mainly into the VRS and perineurium in the perihematomal tissue and ipsilateral brain regions near the hematoma. The soluble blood elements extended more extensively to almost all regions of the brain, including some remote brain areas, such as the contralateral cerebral hemisphere and brainstem. Moreover, the fluorescein dyes were observed in lymph sinuses in the bilateral deep cervical lymph nodes as early as 1 hour after ICH. Lymphostasis, which peaked three days after ICH, was observed in the brain tissues around hematoma. The current findings suggest that the perivascular and perineural extension of hematomas widespread distributes in the central nervous system, and is involved in a series of pathologic processes in ICH, such as the remote effects of a hematoma and lymphatic encephalopathy.

1. Introduction

Spot hemorrhagic lesions occur in perihematomal tissue after intracerebral hemorrhage (ICH). Such lesions usually surround the blood vessels in a ring shape; hence, the name ring hemorrhages (Treip, 1978). Ring hemorrhage is generally considered a secondary lesion that results from microangiopathy (Kikuta et al., 2007). The preliminary results of the present study show that ring hemorrhages that develop after experimental ICH are not caused by microangiopathy, but by the distant extension of blood along the loose tissue spaces, such as perivascular spaces or Virchow–Robin spaces (VRS)

* *Corresponding author at:* Department of Neurology, Nanfang Hospital, 1838 N. Guangzhou Ave, Guangzhou 510515, PR China. Fax: +86 20 62787664.
E-mail address: tianminglu@yeah.net (T. Lü).

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doi:10.1016/j.brainres.2012.02.069

and the spaces around the nerve fibers (perineurium). This occurrence, which is considered perivascular and perineural extensions of hematomas, suggests one of the main pathologic mechanisms of ring hemorrhage and a series of pathologic processes in ICH, such as the remote effect of hematomas (Lü et al., 2005, 2007).

Blood is composed of two main parts, formed elements, which include erythrocytes, leucocytes, and platelets, and soluble elements, such as albumin and electrolytes. Thus far, no study has explored the distribution of these two main blood elements during ring hemorrhage after ICH. In addition, little is known about how the two blood elements are distributed in the deep cervical lymph nodes, which is believed to be the lymph drainage from the VRS after ICH. Thus, the present study hypothesizes that the distribution of formed and soluble blood elements varies during perivascular and perineural extension of hematomas. To investigate the distribution of formed and soluble blood elements after ICH, an ICH rat model was used. Autologous blood was labeled with fluorescein isothiocyanate (FITC), a common marker for formed blood elements in the microcirculation (Seylaz et al., 1999; Tomita et al., 2008; Unekawa et al., 2010), and carboxytetramethylrhodamine (TAMRA)-labeled bovine serum albumin (TAMRA-BSA), a common marker for soluble blood elements (Bingaman et al., 2003; Vogelhuber et al., 2003). The procedure was followed by direct observation of the distribution of these markers at different times during ICH.

2. Results

2.1. FITC-RBC and TAMRA-BSA

In order to identify FITC and TAMRA accurately in the brain sections, it was necessary to observe the patterns among the FITC-labeled erythrocytes (FITC-RBC) and TAMRA-BSA stock soltions before they were mixed with the autologous arterial blood and observed under fluorescence microscopy before and after fixation. The FITC-RBCs formed disk-like shapes under fluorescence microscopy, whether or not fixation was achieved (Fig. 1A). Before fixation with 4% paraformaldehyde, the TAMRA-BSA appeared orange-red and homogeneous; however, after fixation, it became orange-red granules (Fig. 1B).

2.2. Distribution of FITC-RBC

The distribution of FITC-RBCs in the brain may indicate the pattern of formed blood elements during perivascular and perineural extension of hematomas. The FITC-RBCs were extensively distributed in the VRS and perineurium of the perihematomal tissue (Fig. 1C). The FITC-RBCs easily extended into the corpus callosum (Fig. 1D), the VRS in the ipsilateral basal ganglia out of the hematoma, and the ipsilateral cerebral cortex (Fig. 1E). However, the FITC-RBCs were hardly seen around the perineurium of the brain regions far from the hematoma.

Free FITC, green amorphous matter released from disrupted erythrocytes, was morphologically different from the FITC-RBCs. The free FITC was a soluble matter, differed from its granular form wrapped in RBC. Thus, it indicates the distribution of the soluble blood elements rather than that of formed blood elements. It was distributed more extensively in almost all brain regions than the FITC-RBCs, not only in the regions where the FITC-RBCs were distributed (Fig. 1C), but also in the brainstem, which is quite remote from the hematoma (Fig. 1F).

At different times after hematoma formation, the distribution of FITC-RBCs and free FITC was constantly changing. At 1 h, the FITC-RBCs and free FITC extended into the perihematomal tissue (Fig. 1C); free FITC was located in almost every brain region (Figs. 1C, D, and F). After 6 h, yellow-green or yellow-brown fluorescent granules, seemingly formed by phagocytes that engulfed the FITC, appeared in the VRS throughout the brain (Figs. 1G and H).

2.3. TAMRA-BSA distribution

The TAMRA-BSA distribution in the brain indicates the distribution of soluble blood elements during perivascular and perineural hematoma extension. A mass of TAMRA-BSA, which extended along the VRS and perineurium out of the hematoma, was distributed mainly in the perihematomal tissue (Figs. 1I and J). TAMRA-BSA granules were easily observed in the VRS of the ipsilateral (Fig. 1K) and contralateral cerebral cortex (Fig. 1L) as early as 1 h after hematoma formation. Moreover, they spread into remote brain areas, such as the brainstem, via the VRS (Figs. 1M and N). The TAMRA-BSA granules were found to spread within a short distance along the perineurium into the perihematomal tissue (Figs. 1I, J, and O) and corpus callosum (Fig. 1P). Furthermore, the distribution of TAMRA-BSA granules was similar at different times after hematoma formation.

2.4. Distribution of fluorescein dyes

To determine whether the two types of blood elements drained into the deep cervical lymph nodes, which is believed to be the lymph drainage from the VRS, the distribution of fluorescent granules in the deep cervical lymph nodes was observed. The FITC-RBCs and free FITC were located in the deep cervical lymph nodes on both sides as early as 1 h after hematoma formation, which was mainly in the lymph sinuses. However, they were hardly found in the germinal centers of the deep cervical lymph nodes at 1 h after hematoma formation (Figs. 1Q and R). With time, the distribution of the FITC-RBCs decreased, whereas that of the free FITC increased along with the yellow fluorescent granules. They were seemingly formed by the phagocyte-like cells that engulfed the FITC and gradually migrated from the lymph sinuses into the germinal centers (Fig. 1S). However, the identity of the phagocytes needs to be confirmed through histological staining in future studies. In addition, the changes in the TAMRA-BSA distribution patterns and intervals were similar to those of FITC (Fig. 1T). No granular fluorescent substances were found in the brain tissue and bilateral deep cervical lymph nodes in the sham groups.

2.5. Lymphostasis

Dilated VRS with adjacent cerebral edema, indicating lymphostasis, was observed in the perihematomal tissue as