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Neuroscience Letters 226 (1997) 33–36

NEUROSCIENCE LETTERS

The biphasic opening of the blood–brain barrier in the cortex and hippocampus after traumatic brain injury in rats

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Received 20 January 1997; revised version received 19 March 1997; accepted 19 March 1997

Abstract

This study examined the time course of the blood–brain barrier (BBB) opening and correlated this with brain edema formation after a lateral controlled cortical impact (CCI) brain injury in rats. Quantitative measurement of Evans blue (EB) extravasation using fluorescence was employed at 2, 4, 6 h and 1, 2, 3, 4 and 7 days after injury. Brain edema was measured by specific gravity of the tissue at corresponding time points. Two prominent EB extravasations were observed at 4–6 h and 3-day after injury in the injury-site cortex and the ipsilateral hippocampus. Brain edema became progressively more severe over time and peaked at 24 h after injury and began to decline after day 3. These results suggest that there is a biphasic opening of the BBB after CCI brain injury and the second opening of the BBB does not contribute to a further increase in edema formation. © 1997 Elsevier Science Ireland Ltd.

Keywords: Blood–brain barrier; Brain edema; Controlled cortical impact; Traumatic brain injury

Traumatic brain edema is thought to be a mixed form of brain edema with contributions from cytotoxic (cellular) and vasogenic edema. In vasogenic edema, the primary mechanism is the disruption of the blood–brain barrier (BBB) due to endothelial damage, widening of tight junctions or induction of abnormal vesicular transport, all of which subsequently allow components of blood plasma to enter the brain [11]. This results in increased brain edema formation. A better understanding of the occurrence and time course of the BBB breakdown and whether an increase in brain edema formation accompanies the BBB opening is essential to improve present therapeutic interventions. This would also help to establish a window for treatment after traumatic brain injury (TBI).

Although disruption of the BBB has been widely studied in cerebral ischemia [5,9,15] and some TBI models [16,17] there is limited information regarding the time course of BBB permeability to protein-binding tracers such as Evans blue (EB) after lateral CCI brain injury. The aim of the present study, therefore, was to examine disruption of the BBB after CCI brain injury, using quantitative measurements of extravasated EB and correlate this with brain edema formation.

Male Sprague–Dawley rats (300–350 g) were anesthetized with pentobarbital (50 mg/kg, i.p.). Mean arterial blood pressure and arterial blood gas levels were monitored and maintained within physiological limits. Core and cranial temperatures were monitored with rectal and temporalis muscle probes, respectively and maintained at ranges 37–38°C for rectal and 36–37°C for temporalis muscle temperatures with a heating pad and lamp during experiments.

TBI was induced by using a CCI device similar to that developed by Dixon et al. [7] and described earlier [1,3]. After each animal was placed in a stereotaxic frame, a craniotomy in 6 mm diameter was done midway between the bregma and lambda. Each rat in the experimental groups was injured with a 5-mm diameter tip at a velocity of 3.2 m/s and 2 mm deformation while rats in the control group were subjected to the same surgical procedure, including craniotomy, but received no cortical impact.

Permeability of the BBB to EB was evaluated on six control and 48 injured rats at 2, 4, 6 h, and 1, 2, 3, 4, and

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0304-3940/97/$17.00
PII S0304-3940(97)00239-5

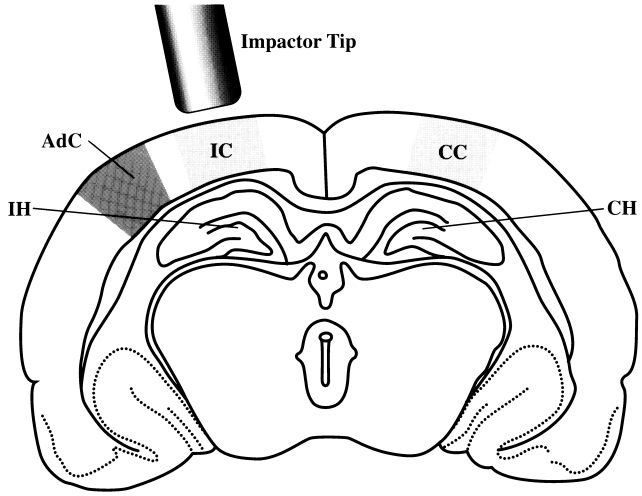

Fig. 1. The placement and site of impact in lateral CCI brain injury in the rat. Regions of dissections after brain injury were the injury site cortex (the left parietal cortex, IC), the cortex adjacent to the injury site (AdC), the contralateral right cortex (CC), and the ipsilateral left hippocampus (IH) and contralateral right hippocampus (CH).

7 days after injury ($n = 6$ for each time point). EB dye (2%), in a volume of 3 ml/kg, was given intravenously (i.v.) and allowed to circulate for 60 min. To remove the intravascular dye, the animals were perfused with saline through the left ventricle. The brains were removed and the sites of greatest injury, that is, the left parietal (injury-site) cortex, the cortex adjacent to the injury site, the contralateral right cortex, and the ipsilateral left and contralateral right hippocampi were dissected (Fig. 1). The method described by Uyama et al. [21] and then applied by others [5,16] was used to evaluate the integrity of the BBB. In this method, a 1 h time period was found to be sufficient for EB to reach brain. Other investigators [5,16] have also given EB 1 h before sacrifice because after observing that EB injected many hours prior to sacrifice has presented contradictory data concerning the time course of BBB breakdown [20]. The administration of EB 1 h before sacrifice provides specific information on BBB permeability especially when early time points have been chosen. The results were expressed as the mean ± SEM μg/g tissue.

Brain edema measurements were undertaken in six control rats and 42 injured rats at 2, 4, 6 h and 1, 3, 4, 5 days. The specific gravity of samples taken from brains at the study sites was measured as an indicator of edema formation [3,4,13]. Tissue samples from each of the five brain regions to be studied were quickly removed in 1-mm^3 pieces and placed in kerosene. A kerosene-bromobenzene continuous gradient column was used for the testing of specific gravities. The values reported reflect the mean of all sections from each region.

A peak increase in permeability to EB was observed in the injury-site cortex, adjacent cortex, ipsilateral hippocampus, and contralateral cortex (in descending order) at 4 h and in all brain regions at 6 h after injury. At 1 and 2 days, the permeability to EB returned to control values in all brain regions but in the injury-site cortex, where significant EB extravasation was still observed (one-factor ANOVA with Bonferroni test, $P < 0.05$; Fig. 2). A second but less prominent increase of EB extravasation was observed at 3 days after injury in the injury-site cortex, adjacent cortex, and the ipsilateral hippocampus. At 4 and 5 days, no significant BBB opening was found.

Increased brain edema corresponds to decreased specific gravity. Brain edema was first seen at 2 h after TBI. A significant increase in brain edema formation was seen at all ipsilateral sites in injured animals at 6 h after injury ($P < 0.05$). The contralateral sites did not show significant changes in brain edema formation at this time ($P < 0.05$). A significant edema formation was found in both ipsilateral

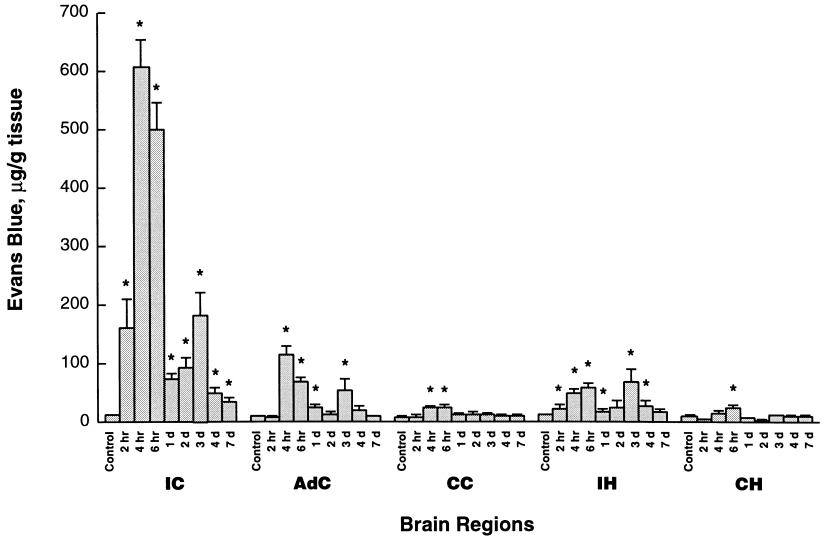

Fig. 2. Measurement of extravasated Evans blue in the injury-site cortex (IC), the adjacent cortex (AdC), the contralateral cortex (CC), the ipsilateral hippocampus (IH), and the contralateral hippocampus (CH) in the injured and control groups at 2, 4, 6 h and 1, 2, 3, 4, and 7 days after brain injury. Data are mean ± SEM. *$P < 0.05$ compared with control animals (one-factor ANOVA with the Bonferroni test).

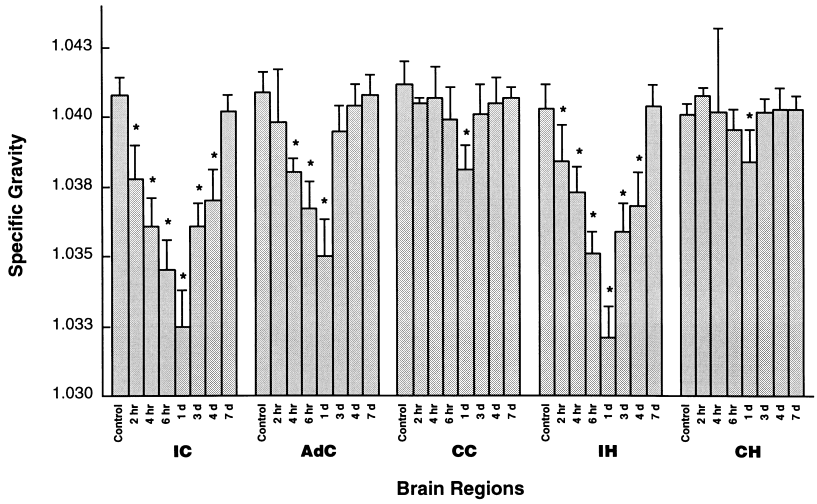

Fig. 3. Specific gravities of the injury-site cortex (IC), the adjacent cortex (AdC), the contralateral cortex (CC), the ipsilateral hippocampus (IH), and the contralateral hippocampus (CH) in the injured and control groups at 2, 4, 6 h and 1, 2, 3, 4, and 5 days after brain injury. Data are mean ± SEM. *$P < 0.05$ compared with control animals (one-factor ANOVA with the Bonferroni test).

and contralateral cortices and hippocampi in the injured animals at 1 day after injury ($P < 0.05$). The brain edema decreased but remained elevated at all ipsilateral sites and totally resolved in the contralateral cortex and hippocampus at 3 days after TBI (Fig. 3).

This study has showed that the BBB opening occurs in a biphasic pattern after CCI brain injury. The first opening appears to be maximum around 4–6 h after TBI. The BBB remained open through 24 and 48 h in injured animals compared to controls. At 3 days, a second prominent opening of the BBB occurred and this increase was significant compared to that at 24 h after TBI ($P < 0.05$). These results are in part consistent with previous studies [5,15]. The biphasic pattern of the BBB opening has been well described in ischemic brain injury [5,15] but it remains to be defined in TBI models. In a recent study [1], a second opening of the BBB has been noted only in the ipsilateral hippocampus but not in the cortex 1 and 2 days after CCI brain injury. Shapira et al. [16] has reported maximum EB extravasation at 4 h after brain injury induced by a weight drop model but they have not observed a second opening up to 7 days post-injury.

Underlying mechanisms leading to the biphasic BBB opening remain speculative. A first possible mechanism is that ischemic and traumatic brain injury are believed to share at least in part a common pathophysiology. A significant fall in cerebral blood flow has been found in the cortex and hippocampus after cortical contusion [6,19]. It is also known that ischemic changes in the brain occur immediately after head injury and persists up to 12 h [12]. Thus, a second opening of the BBB may be related to delayed reperfusion of the ischemic tissues after traumatic brain injury.

A second possible mechanism for a biphasic BBB opening is that the initial severe injury to the brain may result in torn blood vessels and disruption of endothelial membranes which subsequently leads to an early opening of the BBB. The second, delayed opening may result from known mediators of BBB disruption, including arachidonic acid metabolites such as leukotrienes and prostaglandins [4], polyamines [3], histamine [22] and free radicals [8]. Soares et al. [18] recently reported that the role of inflammatory leukocytic recruitment occurs initially in the damaged vasculature and then spreads into the injured cortex and hippocampus. Interestingly they found leukocytic recruitment only in regions showing BBB disruption. Thus, in addition to a primary shear injury that probably contributes to early opening of the BBB, accumulation of leukocytes in the damaged brain regions may contribute to the delayed second opening of the BBB by secreting cytotoxic substrates such as lysozomal enzymes and cytokines. The role of this inflammatory process following TBI has been proposed in delayed deterioration due to increased brain edema following TBI in clinical studies. Recently, Holmin and Mathiesen [10] have demonstrated a secondary increase in brain edema starting on day 5 and continuing through day 7 following an initial peak of edema on day 1 in rats subjected to TBI. In contrast to their study ours and others have not found similar trend up to 5 days after TBI.

In the present study brain edema formation was first seen at 2 h after TBI and localized initially in the brain regions received cortical impact. It then spread through other brain regions remote to the injury-site cortex with a peak increase in all brain regions at 24 h. Early opening of the BBB seems to be an important contributing factor to brain edema formation, which peaks around 24 h after TBI in the present study. A second opening of the BBB at 3 days, however, was not associated with another peak of brain edema, suggesting that the second opening does not contribute to a further increase in edema. A similar finding has been obtained following permanent focal cerebral ischemia by Hatashita and Hoff [9]. They found that a delayed opening

of the BBB after 7 days does not correlate with brain edema formation, which was maximum around 2–3 days following permanent middle cerebral artery occlusion. Thus, a third possible mechanism for the second opening of the BBB after CCI brain injury might be due to re-absorption of edema fluid. Given the brain edema and BBB findings, this may be the predominant mechanism.

Vasogenic edema has long been known to be a major contributor to post-traumatic brain edema [11]. However, it has recently been suggested that the vasogenic component of traumatic brain edema may be overemphasized and that the predominant form of edema might be cellular edema [2,14]. Currently there is no data available to support this hypothesis. Therefore, further studies are necessary to clarify this issue and understand the mechanism underlying the second opening of the BBB.

This study was supported by funding from an NIH grant (PO1 NS 31220) and Department of Veterans Affairs. We carefully adhered to the animal welfare guidelines set forth in the 'Guide for the Care and Use of Laboratory Animals', US Department of Health and Human Services, Pub. No. 85-23, 1985.

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