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Research Report

Blood–arachnoid barrier disruption in experimental rat meningitis detected using gadolinium-enhancement ratio imaging

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ABSTRACT

Disruption of the central nervous system (CNS) barriers is one of the major pathophysiological consequences of bacterial meningitis. The increase in the permeability of the CNS barriers caused by the disruption is thought to contribute to the development of adverse neurological outcomes. We have established a method by which the CNS barrier permeability can be demonstrated by the gadolinium-enhancement ratio (GER) calculated from the T\textsubscript{1} weighted image (T\textsubscript{1}WI) which is based on gadolinium-enhanced magnetic resonance imaging (GdEMRI). The present study examined the disruption of CNS barriers such as blood–brain barrier (BBB), blood–cerebrospinal fluid barrier (BCSFB) and blood–arachnoid barrier (BAB) in rats with meningitis induced by lipopolysaccharide (LPS) or interleukin (IL)-1\textbeta. Four hours after intracisternal injection of LPS or IL-1\textbeta, severe disruption of the BAB, but not the BBB or BCSFB, was observed. This suggests that the BAB, rather than the BBB or BCSFB, plays a key role in the influx of blood-borne cells and substances during meningitis. The BAB is therefore more vulnerable to disruption than the BBB or BCSFB during meningitis in rats. In addition, GdEMRI with GER imaging analysis appears to be useful in spatio-temporal studies on the function of the CNS barriers under various physiological and pathological conditions.

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1. Introduction

Meningitis is the inflammation of the protective membranes known as the meninges in the central nervous system (CNS), which may be caused by infection with bacteria, and occurs in infants, children and adults (Sáez-Llorens and McCracken, 2003). Bacterial meningitis is now a top 10 infectious cause of death worldwide (Grimwood et al., 2000). The mortality rate of bacterial meningitis has remained 5–40% and causes neurological sequelae such as cranial nerve damage with cerebral
infection, learning disabilities and/or mental retardation in up to 30% of the survivors (Mustafa et al., 1990; Quagliarello and Scheld, 1992; Tunkel and Scheld, 1993). The inflammation that occurs in the subarachnoid space (SAS) during bacterial meningitis is not a direct result of bacterial infection but can be attributed to the response of the immune system to the entrance of bacteria into the CNS (Tunkel et al., 1999). When components of the bacterial cell membrane such as lipopolysaccharide (LPS) are identified by the immune cells of the brain such as astrocytes and microglia, they respond by releasing proinflammatory mediators, such as cytokines and prostaglandins. These subsequently lead to an increase in the permeability of the blood–brain barrier (BBB), which then triggers trans-endothelial migration of blood-borne cells such as neutrophils, mononuclear leukocytes, and macrophages, and the leakage of plasma proteins that further lead to inflammation of the meninges, vasogenic cerebral edema, and cytotoxic edema (Tunkel and Scheld, 1993; Pfister et al., 1994; Van Furth et al., 1996). In our previous report, we used Gd-enhanced MRI (GdEMRI) in order to sequentially quantify the permeability of the CNS barriers in experimental rat meningitis induced by LPS and interleukin (IL)-1β (Ichikawa et al., 2010).

In the present study, we have determined the changes in CNS barriers by quantitative analysis of Gd-DTPA leakage into the brain following IC injection with lipopolysaccharide (LPS) or human recombinant IL-1β (hrIL-1β) using GdEMRI and Gd-enhancement ratio imaging (GERI).

## Table 1 – Body temperatures of rats injected intracisternally with LPS and hrIL-1β.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Body temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSF</td>
<td>37.2 ± 0.1</td>
</tr>
<tr>
<td>LPS 200 μg</td>
<td>38.7 ± 0.1</td>
</tr>
<tr>
<td>IL-1β 2.5 × 10^4 U</td>
<td>38.2 ± 0.1</td>
</tr>
<tr>
<td>IL-1β 5 × 10^4 U</td>
<td>38.6 ± 0.0</td>
</tr>
<tr>
<td>IL-1β 5 × 10^4 U + Prednisolone</td>
<td>37.6 ± 0.1</td>
</tr>
</tbody>
</table>

ACSF (5 μl), LPS (200 μg) or hrIL-1β (2.5 × 10^4, 5 × 10^4 U) was injected into the intracisternal cavity of the rats. Prednisolone was administered orally to the rats 30 min before the injection of hrIL-1β (5 × 10^4 U). Four hours after the LPS or hrIL-1β treatment, the rectal temperature of each rat was monitored by a thermometer. The results represent the means ± SE; *P < 0.05, ACSF versus LPS or hrIL-1β, **P < 0.05, hrIL-1β (5 × 10^4 U) versus LPS (200 μg). Gd-DTPA (0.2 mmol/kg) was administered intravenously. After 5 min, the T1WI were monitored. The GERI were calculated from the T1WI.

![Fig. 1](image-url)
meningitis, rats intracisternally (IC) injected with LPS and hrIL-1β can be used as an experimental model for the meningitis. The hyperthermia induced by LPS was equivalent to that by hrIL-1β. Furthermore, the use of prednisolone to clinically treat bacterial meningitis significantly attenuated the hyperthermia. In addition, we examined the infiltration of WBCs into the SAS in these model animals. The infiltration of WBCs into the CSF collected at 4 h after hrIL-1β (2.5×10^4 and 5×10^4 U) injections were 3.15±0.50×10^3/ml and 4.05±0.74×10^3/ml, respectively, although they were detected at less than 1×10^3 cells/ml in the control (ACSF) rats (Fig. 3). Prednisolone also significantly inhibited the infiltration of WBCs into the CSF of the hrIL-1β 5×10^4 U-injected rats (2.64±0.78×10^3/ml) (Fig. 3). These findings indicated that the rats injected with LPS and hrIL-1β develop conditions that mimic the processes in the SAS after bacterial infection causing bacterial meningitis.

2.2. Influence on GERI following injection of LPS or hrIL-1β

In order to determine the effects of injection of LPS or hrIL-1β on the CNS barriers, semi-quantitative analyses were performed using GERI based on GdEMRI. The effects on the BBB, BCSFB and BAB were evaluated by the signal intensity (SI) measured at the cerebral cortex (Cx), lateral ventricle (Lv), and peri-Lv as brain parenchyma, and SAS, respectively. The SI at the peri-Lv and the Cx were not increased by the injection of LPS or hrIL-1β (Fig. 4; K–O, Q, S, U, W). On the other hand, the SI in the Lv (Fig. 4; K–Q, O, S, U, W) and SAS (Fig. 4; K–Q, O, P, R, T, V) were significantly increased at 4 h after the injection of LPS or hrIL-1β. No Gd-DTPA leakage into the brain parenchyma was seen regardless of the high SI in the Lv in rats with experimental meningitis. These results indicated that failure of the BBB and BCSFB were not caused by the experimental meningitis, but that the BAB permeability increased in the SAS.

2.3. Quantitation of CNS barrier breakdown by LPS and hrIL-1β

The dynamic changes in the CNS barriers were quantified by the Gd-DTPA concentrations. In the Lv space, the Gd-DTPA concentrations, 90.1±10.4 μM in the LPS-injected rats, and 128.3±8.6 and 146.5±4.5 μM, in the hrIL-1β-injected rats (2.5×10^4 and 5×10^4 U, respectively) were 4–7 fold higher than that (20.8±7.5 μM) of control (ACSF) animals (Fig. 5). The concentrations of Gd-DTPA in the brain parenchyma of peri-Lv and Cx were not increased in the experimental meningitis models, similar to the results of the semi-quantitative analyses described above. Using the Gd-DTPA tracer, the BBB and BCSFB were found to be impermeable at 4 h after LPS and hrIL-1β injections.
In the SAS, the concentration of Gd-DTPA in the LPS-injected rats was 90.1±10.4 μM, and the concentrations in the 2.5×10^4 and 5×10^4 U hrIL-1β-injected rats were 96.2±4.5 and 122.4±5.9 μM, respectively (Fig. 5). The extent of the BAB disruption after the LPS (200 μg) injection was almost equivalent to that of the hrIL-1β (2.5×10^4 U)-injected rats. Therefore, the data presented here indicate that BAB breakdown, but not breakdown of the BBB or BCSFB, occurred at 4 h after LPS and hrIL-1β injections.

2.4. Effect of prednisolone on the BAB disruption by hrIL-1β

To evaluate the effects of prednisolone, a steroidal anti-inflammatory drug used to treat the bacterial meningitis, on the BAB disruption induced by hrIL-1β (5×10^4 U), the rats were administered oral prednisolone 30 min before the hrIL-1β injection. Prednisolone significantly inhibited the Gd-DTPA leakage into the SAS of rats with experimental meningitis induced by hrIL-1β (vehicle: 122.4±5.9 μM, versus hrIL-1β plus prednisolone; 70.2±7.2 μM) (Fig. 5).

3. Discussion

In the current investigation, we found that LPS and hrIL-1β increased the permeability of the BAB, but not that of the BBB or BCSFB at 4 h after injection in rats.

The mechanisms of pathogenesis of meningitis caused by bacteria are largely unknown. After bacteremia, pathogens have been shown to penetrate into the CSF through the BBB and/or BCSFB to enter the SAS by several mechanisms.
molecules in and out of the CNS. However, little is known about the BAB, which has been the least studied and appears to be the structurally most complex of the CNS barriers (Saunders et al., 2008).

Although the pathogenesis of bacterial meningitis is involved in the dysfunctions of the BBB and BCSFB, it was not known whether the BAB was affected during bacterial meningitis (Tunkel et al., 1990). Sometimes, the BAB was confused with or included in the BBB and/or BCSFB in previous reports. In the present study, we focused on the BAB in the SAS, because the primary target site of bacterial meningitis is the SAS. The BAB is composed of an arachnoid barrier cell layer with numerous tight junctions (TJs), and is considered to represent an effective morphological and physiological meningeal barrier between the CSF in the subarachnoid space and the blood circulation in the dura. The arachnoid barrier layer is always characterized by a distinct continuous basal lamina on its inner surface towards the innermost collagenous portion of the arachnoid (Vandenabeele et al., 1996). Given the complexity of these structures and the small spaces involved, it has been difficult to observe the SAS in living animals. Our previous report showed the possibility of determining BAB dysfunction in the SAS using GdEMRI/GERI (Ichikawa et al., 2010).

In the present study, the Gd-DTPA concentrations in the LV were significantly increased by LPS or hrIL-1β, but there was minimal leakage into the brain parenchyma (Fig. 4). These results suggest that Gd-DTPA may not be able to pass into the CSF of the LV, and the high SI on GERI may be caused by Gd-DTPA in capillary vessels of the choroid plexus. This is because if the Gd-DTPA passes through the BCSFB from the capillary vessels of the choroid plexus, it can diffuse easily into the cerebral parenchyma. Therefore, these findings demonstrate that the BBB and BCSFB are not disrupted at 4 h after LPS or hrIL-1β IC injections (Figs. 4 and 5). Other investigations have reported that the disruption of BBB and BCSFB in these model rats appeared at 6-40 h later (Blamire et al., 2000; Leib et al., 2000). On the other hand, the Gd-DTPA concentrations in the SAS were significantly increased by LPS or hrIL-1β (Figs. 4 and 5). The SAS is surrounded by the arachnoid and the pia mater, having arteries running on the brain surface as well as veins connected to the venous sinus of the dura mater, and the BAB exists in the subarachnoid and the arachnoid barrier cell layers (Peters et al., 1991). These vessels with TJs in the SAS, but without TJs in the dura mater, are surrounded by the leptomeninges (Friede and Schachenmayr, 1978). Although systemically injected Gd-DTPA diffuses into the epidural and intradural spaces, Gd-DTPA is not normally detected in the SAS. Thus, these findings suggest that the disruption of the BAB is firstly involved in the inflammation of the meninges by inflammatory mediators such as LPS or IL-1β in the SAS, and secondary leads to severe damage to BBB and BCSFB and cytotoxic cerebral edema.

While the BAB breakdown was concluded to lead to leakage of Gd-DTPA from the intradural space into the SAS, hyperpermeability of the vessels in the SAS could not be ruled out. In this study, it was not clear which route was involved in the leakage of Gd-DTPA into SAS. It is therefore possible that the blood vessels with TJs and the pia mater of SAS function as CNS barriers. Taken together, we propose that there exists a blood–pia mater barrier (BPMB) or a blood–leptomeninges barrier (BLMB) including the BAB and BPMB as comprehensive

(Gottschalk and Segura, 2000; Kim, 2003). The robust inflammation elicited by bacterial products such as LPS persists after the bacteria are destroyed by the host responses and antibiotic therapy (Kaplan et al., 2004). The LPS produced by meningeal pathogens leads to the production of different inflammatory mediators including IL-1α, IL-6, tumour necrosis factor-α and platelet activating factor, as well as other immune responses, including production of nitric oxide, matrix metalloproteinase-2, and prostaglandins (Ramilo et al., 1990; Pignol et al., 1990; Leib and Tauber, 1999). Cytokines such as IL-1β produced by LPS-stimulated cells are thought to be implicated in the failure of CNS barriers in the meningitis induced by LPS (Jaworowicz et al., 1998; Garabedian et al., 2000).

In the present study, the number of WBCs that penetrated into the CSF increased 4 h after IC injection of hrIL-1β, and prednisolone significantly suppressed the hrIL-1β-induced WBCs penetration into the SAS. The ensuing influx of WBCs and altered CNS barrier permeability results in the release of proteolytic products and toxic reactive oxygen species. CNS barrier failures allow toxic substances to enter the ventricular system and subsequently reach the brain parenchyma. In the present experiments, the model rats used represented these processes of CNS barrier failure after inoculation of bacteria into the CSF of the SAS.

CNS barriers consist of at least the BBB, BCSFB, and BAB, which differ from each other in morphology, constituent cells, locations, and functions (Saunders et al., 2008). As is well known, the BBB and BCSFB are composed of endothelial, choroidal plexus, and glial cells that restrict the traffic of most...
terms. However, to resolve the morphological existence and function of the BPMB or BLMB in the SAS, further ultrastructural and functional studies will be required.

In conclusion, we have demonstrated that disruption of the BAB was present at 4 h after IC injection of LPS or hrIL-1β, but that the BBB and BCSFB were not disturbed in the rat GdEMRI with GERI analyses. These findings suggest that the disruption of the BAB is caused by inflammation of the meninges after acute infection.

4. Experimental procedures

4.1. Materials

Human recombinant IL-1β (hrIL-1β), 2×10^7 units/mg protein) was synthesized at the Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). Lipopolysaccharide (LPS; E. coli serotype 055:B5) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Prednisolone was purchased from Wako Pure Chemical Industries, Ltd. (Tokushima, Japan). Gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA; Magnevist®) was purchased from Bayer Health Care Pharmaceuticals Inc. (Berlin, Germany).

4.2. Induction of experimental meningitis in rats

The protocols for all animal experiments were approved by the Guidelines for Animal Care and Use at Otsuka Pharmaceutical Co., Ltd. Forty-five male Wistar rats (300–350 g) were purchased from Charles River (Tsukuba, Japan) and were used in these experiments. Rats were housed in a room (23.0±1.0 °C) that was lit for 12 h (07:00–19:00) daily. Rats were allowed free access to tap water and pellet food (MF, Oriental Yeast Co., Ltd., Tokyo, Japan). All efforts were made to minimize the number of animals used and their suffering. The rats were anesthetized with Tribromoethanol (Avertin®; 25 mg/kg; Wako Pure Chemical Industries, Ltd., Osaka, Japan) and placed in a stereotaxic frame (SR-5R, Narishige, Japan). A microinjection pump was used to inject 5 μl of artificial CSF (ACSF; containing LPS (200 μg) or hrIL-1β (2.5×10^4 and 5×10^4 U) into the IC cavity over 5 min using a rectal thermocouple probe and a thermostatically controlled pad (BWT-100, Bio-Research Center, Nagoya, Japan).

4.3. Measurements of body temperature in experimental meningitis rats

Four hours after the IC injection of LPS or hrIL-1β, the rectal (core) temperature of each rat was monitored by a thermometer (BWT-100) for 20 s (Fig. 1). The copper-constantan thermocouple as a rectal probe was inserted into the colon about 1 cm beyond the anus.

4.4. In vivo MRI acquisitions

MRI measurements were performed using the Inova 300 Imaging System (7 T) with the VNMRj 1.1d software package (Varian, Inc., Palo Alto, CA, USA). A volume coil and a surface coil (RAPID Biomedical GmbH, Germany) were used for signal transmission and detection, respectively. During MRI measurements, a rat was anesthetized by inhalation of 1–1.5% halothane in a N2/O2 (70:30) mixture gas via a facemask. During the MRI measurements, the body temperature was measured using a rectal thermocouple and it was kept constant with a feedback-controlled warm-water pad (Yamashita Tech System, Tokushima, Japan) connected to a rectal probe (Photon Control Inc., Burnaby, BC, Canada). Four hours after each treatment, Gd-DTPA at a dose of 0.2 mmol/kg was administrated intravenously. Acquisition of Gd-enhanced MRI (GdEMRI) images was obtained before, and at 5 min after the administration of Gd-DTPA (Fig. 2). The T1 weighed images (T1WIw) were acquired by a 2-dimensional multi-slice spin echo sequence using the following parameters: repetition time (TR) 500 ms; echo time (TE)=10 ms; number of scans=2; slice thickness=2 mm; matrix size=256×256; the images were zero-filled to 512×512; and the field of view was 25×25 mm.

4.5. Imaging of GER

The GERI was calculated from the MRI without Gd-DTPA and GdEMRI according to the formula (Fig. 2) (Erwin et al., 2002; Ichikawa et al., 2010):

\[
\text{GER} = \frac{T_1\text{WIw}/(\text{Gd-DTPA}) - T_1\text{WIw}/(\text{Gd-DTPA})}{T_1\text{WIw}/(\text{Gd-DTPA})} \times 100.
\]

4.6. Measurements of signal intensities in the region of interest

To evaluate the disruption of the CNS barriers from GERI, the region of interest (ROI) was selected in the LV, the peri-LV and the Cx as brain parenchyma, as well as the SAS space (Fig. 4C; Arrows 1, 2, 3 and 4). The SI in the ROI was measured at 5, 15, 30 and 60 min after injection with Gd-DTPA. The SI reached the highest at 5 min, and then decreased 30% at 15 min. After 30 min, the SI by Gd-DTPA was not detectable in SAS. Each SI in the ROI was measured for the GERI using the Image Brawer software program (Varian, Inc., Palo Alto, CA, USA).

4.7. Measurements of the Gd-DTPA concentration

The quantitative assessment of the Gd-DTPA concentrations in the brain regions was calculated from the SI of GERI according to a previously described method (Ichikawa et al., 2010). A linear correlation between the signal intensities of GERI and Gd-DTPA concentrations (0–150 μM) was calculated. This relational formula showed that the GdER value can be used to quantitatively evaluate the Gd-DTPA concentration.

4.8. Administration of prednisolone in hrIL-1β-induced meningitis rats

Prednisolone was suspended in 0.5% carboxymethyl cellulose (CMC) at a concentration of 0.6 mg/ml. The solution was...
administered orally to the rats 30 min before the IC injection of artificial CSF containing hrIL-1β (Fig. 1). The CMC (0.5%) solution was administered to the control group.

4.9. Infiltration of white blood cells into the CSF

The CSF was collected by aspiration from the cisterna magna via puncture of the allanto-occipital membrane at 4 h after the hrIL-1β injection (Fig. 1). The CSF samples were used to count white blood cells (WBCs) by the trypan blue exclusion method (Tunkel and Scheld, 1993).

4.10. Statistical analysis

The results were expressed as the means ± SE. The statistical analyses were performed using the SAS® software package (SAS Institute, Japan). The differences between the treated and control groups were assessed by one-way ANOVA followed by the two-tailed Dunnett’s test, with statistical significance set at P<0.05.

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