Gelatinase B modulates selective opening of the blood-brain barrier during inflammation

SHEILA MUN-BRYCE AND GARY A. ROSENBERG
Departments of Neurology and Cell Biology and Physiology, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

Mun-Bryce, Sheila, and Gary A. Rosenberg. Gelatinase B modulates selective opening of the blood-brain barrier during inflammation. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1203–R1211, 1998.—Matrix metalloproteinases (MMPs) are associated with neuroinflammatory diseases, and blood-brain barrier damage is a pathophysiological consequence of central nervous system inflammation. We examined whether an increase in MMP production is coupled with the breakdown of blood-brain barrier integrity in the lipopolysaccharide (LPS)-injured brain. Rat brain stimulated with LPS showed a significant rise in gelatinase B (MMP-9) production at 24 h compared with either tumor necrosis factor-α (TNF-α) or saline-injected controls. Latent 92-kDa gelatinase B was detected by 4 h, peaked at 8 h, and persisted for 24 h after LPS injection. Production of the active 84-kDa form of gelatinase B was less pronounced, but paralleled 92-kDa enzyme expression. Breakdown in blood-brain barrier integrity, measured by the infiltration of radiolabeled exogenous markers into the brain, was significant to [14C]sucrose (molecular mass 342 Da) and [14C]dextran (molecular mass 50–90 kDa) molecules in LPS-injected animals compared with saline-injected controls. The extent of MMP involvement in barrier permeability was examined in animals treated with the MMP inhibitor BB-1101. A significant drop in gelatinase A and B production was detected in LPS-injured animals receiving BB-1101 compared with untreated animals. This MMP inhibitor also reduced [14C]sucrose uptake in LPS-injected animals, but had no effect on [14C]dextran uptake. MMP production is upregulated in LPS-injured brain tissue and is instrumental in regulating the size-differentiated opening of the blood-brain barrier during acute neuroinflammation.

basal lamina; lipopolysaccharide; matrix metalloproteinases; metalloproteinase inhibitor; neuroinflammation

CENTRAL NERVOUS SYSTEM injury or infection triggers a host immune response that generates the early synthesis and release of inflammatory cytokines, initiating a series of immunological events that results in the recruitment of lymphocytes and neutrophils (17). Two cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), have been associated with disruption of the blood-brain barrier, which is a key pathological feature of multiple sclerosis (30) and bacterial meningitis (25). The proteolytic activity of the matrix metalloproteinases (MMPs) has been implicated in the acute opening of the blood-brain barrier when brain tissue is stimulated with TNF-α (28). Lipopolysaccharide (LPS), which consists of cell wall components of gram-negative bacteria, is a potent stimulator of cytokines and has been linked to the pathological opening of the blood-brain barrier after injection into the cerebrospinal fluid (34). Therefore, in this study we hypothesized that in vivo MMP production mediates the LPS-induced blood-brain barrier opening.

The blood-brain barrier shields the brain from systemic fluctuations in the blood by regulating the exchange of substances between the vasculature and the central nervous system. This physiological barrier is composed of tight-junctioned capillary endothelial cells, supported by a microenvironment of astrocytic processes, pericytes, and microglial cells (26). Proteases disrupt the blood-brain barrier by degrading the basal lamina, which separates the brain endothelial cells from adjacent pericytes and astroglial cells. Damage to the blood-brain barrier leads to the influx of fluid and proteins into brain tissue, resulting in tissue swelling and neuronal destruction (5).

MMPs are a gene family of zinc- and calcium-dependent endopeptidases that cleave components of the extracellular matrix (ECM). Secreted in an inactive zymogen form, they are activated in the ECM by different mechanisms (4). Although there is a significant structural homology at the amino acid level among the members in this proteolytic family, they differ in the specific ECM substrates they degrade. MMPs contain a highly conserved sulfhydryl side chain in the NH2-terminal propeptide domain, which is responsible for maintaining the latent form of the zymogen. This propeptide side chain has a cysteine residue that binds to the catalytic zinc ion in the active site of the enzyme (31). Disruption of the cysteine-zinc bond causes a conformational change that results in its activation. Once the propeptide fragment is cleaved, a stable active enzyme is formed. Gelatinase A (72-kDa type IV collagenase, MMP-2) and gelatinase B (92-kDa type IV collagenase, MMP-9) specifically degrade type IV collagen (18), a key structural component of the basement membrane that surrounds blood vessels. Although gelatinase A and B cleave similar substrates, they are separate gene entities and are differentially regulated at the transcriptional level (33).

We have shown that an imbalance between proteinases and their inhibitors modulates blood-brain barrier function. Activated gelatinase A increased blood-brain barrier permeability, which was attenuated by an injection of human recombinant tissue inhibitor of metalloproteinase-2 (29). The simultaneous upregulation of gelatinase B and increased uptake of the small molecule sucrose in the TNF-α-injected brain was significantly reduced with a synthetic MMP inhibitor (28). We now report that LPS induces MMP production and activation, which is coupled with the size-specific opening of the blood-brain barrier. Initial experiments using gelatin-substrate zymography delineated the type of MMPs that are produced in brain tissue stimulated...
with an intracerebral injection of the inflammatory mediators TNF-α or LPS. Changes in MMP production were monitored over the initial 24 h after LPS injection. Blood-brain barrier permeability to different sized molecules was characterized in the LPS-stimulated brain in the presence of a hydroxamate-based metalloproteinase inhibitor, BB-1101.

METHODS AND MATERIALS

Intracerebral injection of inflammatory mediators. Male Sprague-Dawley rats, weighing 265–320 g, were initially anesthetized with 2% halothane in 70% nitrous oxide and 30% oxygen. The halothane dose was decreased to 1.2% once surgery commenced. Intracerebral injections were performed as described in detail (27). The animals were positioned in a stereotactic headholder (Kopf Instruments, Tujunga, CA), and Burr holes were made in the skull of both hemispheres, 3 mm from midline, at the bregma. Bilateral intracerebral injections of LPS in 5 µl sterile saline (0.10 µg; Sigma, St. Louis, MO), TNF-α in 5 µl sterile saline (10^4 U; UBI, Sybracuse, NY), or 5 µl of 0.9% sterile saline was administered 5 mm below the dura, using a 23-gauge infusion needle, over 5 min. Evan’s blue dye was added to the injectates for localization of the injection site. After the infusion, the burr holes were sealed with bone wax and the incision was sutured.

Twenty-four hours after the intracerebral injections, the animals were anesthetized with pentobarbital sodium (60 mg/kg ip) and killed with a potassium chloride injection into the heart. The brain was excised and dissected under sterile conditions. Tissue samples of the injury site included the caudate/putamen region and weighed 100–200 mg. Samples were stored at −70°C for assay of MMPs by gelatin-substrate zymography.

To characterize the time course of metalloproteinase production in the LPS-stimulated brain, animals were surgically prepared as described. The animals received bilateral intracerebral injections of LPS or saline before or after TNF-α or LPS injection. At time = 2, 4, 6, 8, 12, and 24 h, the animals were killed and brain tissue samples were collected using the above procedures.

The animal protocol was approved by the Animal Research Committee at the University of New Mexico and conformed to the National Institutes of Health guidelines for use of animals in research.

Zymography assay of metalloproteinase production. The production of metalloproteinases in LPS-injected animals was assayed using the SDS-PAGE gelatin-substrate zymography method (27). Brain tissue samples were minced and solubilized in 0.5% Triton X-100 in 20 mM phosphate-buffered saline (pH 7.0), for a final concentration of 400 mg/ml, for 24 h in 4°C. The supernatant was collected and diluted 1:2 with sterile distilled water and stored at −70°C for assay of MMPs by gelatin-substrate zymography.

To characterize the time course of metalloproteinase production in the LPS-stimulated brain, animals were surgically prepared as described. The animals received bilateral intracerebral injections of LPS or saline before or after TNF-α or LPS injection. At time = 2, 4, 6, 8, 12, and 24 h, the animals were killed and brain tissue samples were collected using the above procedures.

The animal protocol was approved by the Animal Research Committee at the University of New Mexico and conformed to the National Institutes of Health guidelines for use of animals in research.

Zymography assay of metalloproteinase production. The production of metalloproteinases in LPS-injected animals was assayed using the SDS-PAGE gelatin-substrate zymography method (27). Brain tissue samples were minced and solubilized in 0.5% Triton X-100 in 20 mM phosphate-buffered saline (pH 7.0), for a final concentration of 400 mg/ml, for 24 h in 4°C. The supernatant was collected and diluted 1:2 with sterile distilled water and stored at −70°C. Five microliters of diluted supernatant was combined with 3 µl 1.5 M Tris buffer, pH 8.8, and 2 µl SDS gel loading buffer (55% distilled water, 12.5% 0.5 M Tris-HCl, pH 6.8, 10% glycerol, 20% SDS in a 10% wt/vol solution, and 2.5% bromphenol blue in 0.05% wt/vol solution) and loaded onto a prepared gel. Protein standards (GIBCO Life Technologies, Gaithersburg, MD) were run on each gel to verify protein molecular weights. Conditioned media from the HT1080 fibrosarcoma cell line, a known source of metalloproteinases, were also electrophoresed in each gel to verify the gelatinase A and B lysis zones. Gelatin (Sigma, St. Louis, MO) was copolymerized into 10% polyacrylamide gels. All samples, except for the MMP inhibitor study samples, were run on a double-sided 15 × 17-cm vertical gel electrophoresis apparatus (GIBCO Life Technologies). Samples from the MMP inhibitor study were run on a mini-gel 8.0 × 7.3-cm apparatus (Bio-Rad Laboratories, Hercules, CA).

After electrophoresis, the gels were gently agitated in 2.5% Triton X-100 for 15 min. The gels were then incubated at 37°C to allow for enzyme degradation of the gelatin matrix. Gels from the preliminary experiments detecting MMP production in saline-, TNF-, or LPS-injected animals were incubated for 24 h. Gels in all other studies were incubated for 72 h to allow for detection of the 84-kDa active form of gelatinase B. Preliminary experiments depicted a linear increase in all band intensities that was within the range of quantification, when incubation periods were increased from 24 to 72 h at 37°C (data not shown). The gels were incubated in 0.05 M Tris, pH 7.6, 0.2 M NaCl, 0.005 M CaCl2, 0.02% wt/vol Brij-35. To visualize regions of lysis, gels were then stained for 1 h with Coomassie (0.125% Coomassie G-250 wt/vol, 50% methanol vol/vol, and 1% acetic acid vol/vol), then destained in 10% acetic acid for 5 days. Proteolytic bands appeared clear on the blue-stained background.

The gels were then dried between cellulose film and scanned (HP Scanjet et al., Hewlett-Packard) into a Macintosh Power PC computer. Computer image analysis was performed using the electrophoretic gel lane calculation option on the National Institutes of Health Image V.1.56 software program. Gelatinase activity values were obtained by comparatively measuring the lysis zone area of each band on an individual substrate gel. Image density was standardized with an optical density step tablet, which controls for scanning differences. Protein content of the sample supernatants was assayed with the micro bichiorchonic acid method (Pierce, Rockford, IL). Gelatinolytic activity values were normalized by dividing the area of the lysis zone band by the amount of protein.

Activity standards for gelatinase B and A are not available; therefore, quantitation of gelatinase activity levels are expressed as a relative and unitless value. Relative activity values are expressed as the amount of gelatinase activity per protein content in the tissue sample.

Blood-brain barrier permeability measurement in LPS-injected brain. Ten minutes before euthanasia, the animals were anesthetized and either 55% [3H]sucrose, molecular mass 34,000 Da, or [14C]dextran, molecular mass 50–90 kDa (10 µCi; DuPont-NEN, Boston, MA), was administered into the femoral vein. Blood-brain barrier permeability alterations were examined using a modification of the Ohno et al. (23) technique. At the time of death, a blood sample was collected over a 1-min period and the animals were euthanized with potassium chloride. Blood-brain barrier permeability alterations were examined using a modification of the Ohno et al. (23) technique. At the time of death, a blood sample was collected over a 1-min period and the animals were euthanized with potassium chloride. The brain was isolated and frozen in cold 2-methylbutane. An −50-mg tissue sample was dissected from the injection site at the caudate/putamen in each hemisphere and solubilized (500 µl Protosol; DuPont-NEN) overnight at 50°C. Plasma and tissue samples were prepared for liquid scintillation counting (Beckman Instruments, Fullerton, CA) in 4 ml Aquasol (DuPont-NEN).

Capillary permeability was determined by using the radioactivity values to calculate a brain-to-blood ratio. This ratio characterizes the brain uptake of either isotope under experimental conditions compared with saline controls. The assayed values were expressed as disintegrations per minute radioactivity in the tissue sample per milligram tissue sample divided by disintegrations per minute radioactivity in the plasma sample.

Metalloproteinase inhibition in the LPS-injected brain. Four hours after bilateral LPS or saline intracerebral injection, a group of experimental animals received a 30 mg/kg ip injection of the synthetic metalloproteinase inhibitor BB-1101 (British Biotechnology, Oxford, UK). BB-1101 was prepared...
RESULTS

Regulation of MMPs by inflammatory mediators

Zymograms of the TNF-α and LPS-infused brain tissue samples revealed a significant increase in lysis zone at 92 kDa, compared with saline samples (Fig. 1A). This 92-kDa lysis zone corresponded to the known lysis band of gelatinase B in HT1080 fibrosarcoma conditioned media. The 72-kDa lysis zone, corresponding to gelatinase A, was constitutively expressed in all samples. A greater than sevenfold increase in 92-kDa relative activity in the LPS-injected brain tissue samples (12.42 ± 1.24) was seen compared with saline controls (1.67 ± 0.93). Moreover, the intensity of the 92-kDa lysis band was significantly higher in the LPS-injected brain samples than in the TNF-α-stimulated tissue (6.06 ± 1.27). Thus LPS was a more potent stimulator of gelatinase B production than TNF-α at 24 h postintracerebral injection.

The time course of gelatinase B production during the initial 24 h after LPS stimulation is depicted in Fig. 2, A and B. Negligible gelatinase activity at 92 kDa was detected 2 h after LPS or saline injection compared with the constitutively unchanged production of gelatinase A. The 92-kDa lysis band was clearly visible in both control and experimental groups 4 h after injection, with relative activity measurements of 8.76 ± 2.0 in LPS samples, compared with 0.995 ± 0.48 in saline samples. By 6 h, definite lysis activity in the 92-kDa band was present in both the saline (5.37 ± 1.18) and LPS (17.09 ± 1.69)-injected brain tissue samples. Maximal lytic activity at the 92-kDa band (18.17 ± 2.27) occurred at 8 h after LPS injection, falling to 16.54 ± 1.74 by 12 h. Elevated levels of 92-kDa activity (7.27 ± 1.37) persisted at 24 h after LPS infusion. In saline-injected brain tissue, the intensity of the 92-kDa band dropped to 0.90 ± 0.14 at 24 h, which was similar to the level of 92-kDa band intensity, in saline-treated animals at 2 h.

In contrast to the early appearance of the 92-kDa gelatinase, the 84-kDa lysis band was not detected in samples until 6 h after LPS intracerebral injection (Fig. 3). This 84-kDa gelatinolytic band increased in intensity at 8 h and persisted for 24 h post-LPS injection. No 84-kDa lysis activity was detected in saline-injected animals at any observed time points (data not shown). The lysis activity of the 84-kDa band paralleled the fluctuations in the 92-kDa lysis band over the initial 24 h post-LPS intracerebral injection.

LPS modulation of blood-brain barrier permeability

The LPS-induced synthesis of gelatinase B enzyme corresponded to the progression of blood-brain barrier permeability during the initial 24 h postinjection. Figure 4 shows that the intracerebral injection of LPS caused a significant increase in [14C]sucrose uptake into the brain at 4 h (2.49 ± 0.14%) compared with saline controls (1.68 ± 0.08%). Significant increases in [14C]sucrose persisted in brain tissue samples at all time points after LPS injection compared with saline. Maximal [14C]sucrose uptake values were observed at
12 h post-LPS injection (6.1 ± 0.47%) and were significantly higher than [14C]sucrose uptake values measured in control animals (1.65 ± 0.19%). By 12 h, the [14C]sucrose uptake value in saline-injected animals had returned to the 4-h control uptake level (1.68 ± 0.08%).

Brain uptake of [14C]dextran into the brain at 12 h was significantly higher in LPS-stimulated brain tissue (0.75 ± 0.042%) compared with saline (0.50 ± 0.03%), as shown in Fig. 4B. The percentage of [14C]dextran uptake into the brain was comparatively less than [14C]sucrose uptake as determined at the same time point, suggesting that the altered permeability in the LPS-injected brain is selective and detection of these changes is dependent on the molecular size of the radiolabeled marker.

LPS-induced proteolysis alters blood-brain barrier permeability. To determine whether the increased permeability at the blood-brain barrier interface was secondary to LPS-induced metalloproteinase activity, an experimental group of animals was given a synthetic inhibitor of metalloproteinases. Four hours after an intracerebral LPS injection, animals received an intraperitoneal injection of the synthetic MMP inhibitor BB-1101. MMP production and blood-brain barrier permeability were measured 12 h after LPS or saline injection.

A representative zymogram in Fig. 5A depicts the differences in the gelatinolytic intensities of the 92-, 84-, and 72-kDa bands in LPS-injected tissue samples that were either treated or untreated with BB-1101. Figure 5B depicts the statistically significant drop in 92-kDa collagenase production when LPS-injected ani-
Fig. 6, blood-brain permeability to $[^{14}C]$sucrose dropped 4.25 ± 0.22% after treatment with BB-1101. Saline-injected brain samples showed no significant change in permeability to sucrose in either treated (2.05 ± 0.14%) or untreated (1.65 ± 0.19%) animals.

Because blood-brain barrier permeability to $[^{14}C]$dextran was also significantly elevated in LPS-injected animals, the percentage of $[^{14}C]$dextran uptake into the brain was measured in the presence of BB-1101 at 12 h after LPS injection. Brain tissue samples showed no statistical difference in percent dextran space in LPS-injected animals treated with BB-1101 (0.72 ± 0.03% compared with 0.75 ± 0.04%). In summary, BB-1101’s inhibition of gelatinolytic activity (Fig. 5B) was associated with a substantial drop in barrier permeability to small molecule sucrose, but had no effect on the movement of larger molecule dextran across the blood-brain barrier (Fig. 6).

**DISCUSSION**

The intracerebral presence of the inflammatory mediator LPS caused a significant rise in MMP production by 4 h, persisting at 24 h postinjection. Gelatin-substrate zymography revealed a significant elevation in gelatinolytic activity of 92 kDa, which corresponds to the latent form of gelatinase B, in LPS-injected tissue samples at 24 h compared with TNF-α and saline-injected brain samples. Gelatinolytic activity at 72 kDa, which corresponds to the latent form of gelatinase A, was unaffected by either LPS or TNF-α intracerebral injection. LPS induces the release of several inflammatory mediators, including TNF-α, IL-1β, and platelet activating factor, and each of these inflammatory factors can trigger MMP production (4). Gelatinase production was significantly greater after LPS stimulation than after TNF-α, suggesting the involvement of multiple factors and inflammatory mediators in response to the intracerebral injection of LPS.

Time course studies of gelatinase B production in LPS-stimulated rat brain revealed a steady and significant rise in the 92- and 84-kDa forms of gelatinase B at 6 h after injection. This rise persisted for 24 h. Elevated levels of gelatinase B paralleled the observed increase in brain uptake of both sucrose and dextran in the LPS-stimulated brain, indicating a link between MMP production and blood-brain barrier damage. The small increase in MMPs seen at 2 h postinjection of either saline or LPS is most likely due to tissue damage caused by the infusion needle. Damage to brain cells or the surrounding ECM is known to elicit the release of some of the same inflammatory factors induced by LPS in the absence of an antigenic pathogen (12) and can also result in the activation of the immunogenic phenotype in microglia (1).

Increased 92-kDa gelatinase B activity was observed 4 h after LPS injection, although the active 84-kDa form of gelatinase B was not seen until 6 h. Because a latent metalloproteinase enzyme can be transformed into an active configuration without autodeactivation of its propeptide domain (4), active gelatinase B may have been present in the 92-kDa band. The 84-kDa active...
form of gelatinase B was coupled with increased 92-kDa gelatinolytic band intensity. Although sucrose uptake measurements were not obtained in blood-brain barrier experiments at 6 h, measurements before and after this time suggest a continuous increase in sucrose uptake, which is consistent with the gradual rise in relative lysis activity of both the 92- and 84-kDa forms of gelatinase B.

Peak gelatinase B activity was seen at 8 h postinjection, whereas maximal sucrose molecule uptake in the brain was detected 12 h after LPS injection. In fact, a decreasing trend in gelatinolytic activity was seen 12 h after LPS injection when barrier permeability to sucrose steadily increased to its highest levels at this same time point. This latency between MMP production and barrier permeability indicates a delayed MMP proteolytic effect on barrier permeability. The half-life of interstitial collagenase (MMP-1) is reportedly 6–12 h (4). The accumulation of 92- and 84-kDa gelatinase B protein at 8 h after LPS injection may have resulted in a delayed increase in capillary permeability at 12 h.

Production of 92-kDa gelatinase B appears to exceed that of the 84-kDa active form, which supports the belief that MMP activation is tightly regulated.

Size-dependent attributes of the disrupted barrier were examined by monitoring the uptake of dextran into the brain at 12 h post-LPS infusion. Dextran uptake was significantly elevated in brain tissue samples stimulated with LPS, compared with saline, but the overall movement of dextran across the blood-brain barrier was less in both experimental groups than the amount of sucrose infiltration. This finding suggests that an intracerebral injection of LPS precipitates...
tates a size-dependent increase in brain capillary permeability.

Two in vivo brain injury models produce a transient opening of the blood-brain barrier, which is apparently regulated by distinct mechanisms (20). Clearance of different-sized dextran molecules was dependent on molecular size when capillary permeability was osmotically increased (2). In comparison, there was no difference in the clearance of various-sized molecules in the brain of hypertensive animals after an acute insult (11). Altered capillary permeability in the hypertensive model is thought to be due to increased pinocytosis in brain vessel endothelium (22). Vesicular movement of substances across the blood-brain barrier is independent of the size of the molecule. In contrast, the size-dependent movement of solute in the osmotically damaged barrier is more consistent with a pore-related bulk flow mechanism rather than vesicular movement through the endothelial cell (37). The pores are postulated to open when a hyperosmotic solution causes brain endothelial cells to shrink and gaps form in the normally continuous tight junctions.

In our LPS-injury model, the discrepancy in the amount of dextran uptake versus that of sucrose may be best explained by solute transport through injury-induced pores. The percentage of sucrose molecule permeability in our barrier studies was eightfold higher than the percentage of dextran molecule permeability measured in LPS-stimulated brain tissue, which was similar to the clearance data reported by others (20).

Interestingly, BB-1101 treatment showed selective inhibition of uptake of the smaller sucrose molecule into brain tissue, but demonstrated no effect on the larger dextran uptake. Although this further demonstrates the size-dependent attribute of LPS-induced blood-brain barrier opening, it is not consistent with what is known about the barrier closure rate in the osmotically injured animal. According to in vivo studies (37), the rate of closure of the blood-brain barrier in the osmotically induced barrier permeability model also appears to be regulated by a size-selective mechanism. The amount of 79-kDa dextran permeability declined more rapidly than the amount of 342-Da sucrose permeability in the brain after an osmotic insult. A pronounced restriction of larger molecules occurred minutes after the onset of osmotically induced barrier opening compared with small molecules during barrier reclosure, suggesting that the larger pores may be closing faster than smaller-sized pores. BB-1101 appears to selectively inhibit smaller pores in the compromised blood-brain barrier compared with the larger pores, resulting in a decrease in sucrose uptake into the brain while dextran uptake is unaffected.

The neural microenvironment associated with the brain capillary endothelial cells is another vital factor regulating net entry of exogenous substances across the blood-brain interface. Endothelial cells, the surrounding basal lamina, astrocytes, pericytes, and microglia restrict movement of substances across the blood-brain barrier (26). The basal lamina of brain blood vessels is believed to function as an adhesive and supportive substrate for the intimate association of astrocytes and other glial cells with capillary endothelial cells. This lamina is composed of ECM components, including type IV collagen, laminin, fibronectin, and heparin sulfate (36), and can mediate a local inflammatory response (12). Inflammatory mediators such as TNF-α, have been shown to induce gelatinase production resulting in the degradation of fibronectin in the ECM surrounding the microvascular endothelium (24). In addition, proteinases that degrade the ECM have been shown to convert barrier vessels into permeable, fenestrated vessels (14).

Before blood-brain barrier maturation in postnatal rats, increased brain capillary permeability has been attributed to a rise in transendothelial transport activity (35). Electron microscopic studies show vesicles containing ferritin tracer are taken up by pericytes, which are in close contact with the basal lamina in cerebrovasculature of the developing corpus callosum. The pericytes seem to sequester substances that manage to cross the endothelium (8, 35). A crucial function of the basal lamina at the blood-brain interface may be to position and anchor phagocytic cells so that they can intercept blood-borne substances that have managed to penetrate the endothelial cell layer. Degradation of the basal lamina by MMPs in the LPS-induced injury model may result in the breakdown of structural support, compromising the physical association of these barrier components, and interfere with the sequestering of sucrose or dextran molecules by pericytes and other phagocytic cells. Although disruption of cerebrovascular tight junctions was not detected in electron micrographs of collagenase-treated brain samples (29), opening of tight junctions is hard to demonstrate in electron microscopic studies unless an electron-dense tracer, such as lanthanum nitrate, is used (13).

The hydroxamate metalloproteinase inhibitor BB-1101 differentially reduced gelatinolytic activity of the three MMP species detected by gelatin-substrate zymography of brain samples collected 12 h post-LPS stimulation. BB-1101 reduced the lysis area intensity of the 92-kDa band by 54%, the 72-kDa band by 68%, and the 84-kDa band by 73%, suggesting that this hydroxamate inhibitor is exerting its effect at several levels of MMP expression. Hydroxamic acid-based metalloproteinase inhibitors act on active enzymes in which the catalytic zinc atom is free, when the cysteine-zinc interaction at the active site of the enzyme is disrupted (7). In addition, hydroxamate derivatives act on other substrates. Recent studies demonstrated that hydroxamate inhibitors can hinder the conversion of precursor 26-kDa TNF-α to its active 17-kDa form (9, 21). As described earlier, LPS is a strong stimulator of TNF-α synthesis and release. The presence of an MMP inhibitor in an LPS-stimulated animal prevented the accumulation of TNF-α in the blood (9). As induction of MMP gene expression is enhanced by TNF-α-dependent transcription factors (6), the downregulation of mature TNF-α would result in decreased MMP synthesis. There-
fore, the drop in 92-kDa band intensity in BB-1101-treated, LPS-injected animals did not return to saline control levels is indicative that other mechanisms influence MMP synthesis and activation and regulate blood-brain barrier permeability. There are many other factors involved in the acute opening of the blood-brain barrier during cerebral inflammation (3). We showed in an earlier study that sucrose clearance in TNF-α-stimulated brain tissue could be regulated by another related MMP inhibitor when given in either a single- or double-dose regimen (28). An initial intraperitoneal injection of MMP inhibitor, batimastat, given at the time of TNF-α injection and again at 6 h, demonstrated a similar decrease in brain tissue uptake of sucrose, as a single dose of batimastat given 6 h after TNF-α injection compared with control animals. The daily administration of another hydroxamic MMP inhibitor, GM 6001, at the onset of experimental allergic encephalomyelitis resulted in the suppression of clinical inflammatory demyelination (10). Thus the inhibitory effect of hydroxamic MMP inhibitors seems most beneficial when given at the onset of experimental brain injury but can have an effect on barrier permeability when given several hours after injury.

Our studies indicate that increased blood-brain barrier permeability to small and large molecules can be induced by an intracerebral injection of LPS. This increase in barrier permeability correlated with an upregulated production of active and latent gelatinase B protein during the initial 24 h after LPS injury. A marked drop in both forms of gelatinase B production was detected when LPS-injected animals were treated with a synthetic inhibitor of metalloproteinases. This correlated with a decrease in barrier permeability to small molecules but did not affect barrier permeability to large molecules, implicating a size-dependent mechanism of blood-brain barrier opening in the LPS-injection model. These results also suggest that the early administration of an MMP inhibitor within the first 4 h of nervous system infection or injury may lessen blood-brain barrier permeability to blood-borne toxins and the accumulation of edema in the neuronal environment. The LPS-injury model appears to be a significant tool for examining the in vivo mechanisms that regulate gelatinase B production and activation.

Perspectives

Neuroinflammation is an integral component in many nervous system diseases, including multiple sclerosis, brain trauma, stroke, and infection. Recruitment of immune cells into the affected brain region, which is a primary neuroinflammatory response, can damage neuronal tissue and result in debilitating neurological sequelae. For example, despite treatment with antibiotics, the morbidity rate for a central nervous system infection remains ~30% (25). An increase in blood-brain barrier permeability and brain edema commonly occurs as a result of inflammation in the nervous system. MMPs are released during the neuroinflammation in response to cytokines (28). Increased production of these matrix-degrading enzymes has been linked to physiological disruption of the blood-brain barrier.

Previous studies showed upregulation of latent MMP proteins during inflammation in the brain. The present study revealed an active form of gelatinase B at 84 kDa, which is the first in vivo demonstration that the latent 92-kDa enzyme is being converted into its active enzyme form in brain. More importantly, the decrease in blood-brain barrier permeability was linked to a fall in both active and latent gelatinase production in the animals treated with an MMP inhibitor, suggesting a possible therapeutic intervention against the potentially damaging processes of neuroinflammation. The determination of which cell types are producing these and other MMPs at the gene and protein level would further our understanding of the mechanisms that modulate the expression of these enzymes during a neuroinflammatory event.

These studies were done in partial fulfillment of the PhD degree requirements for S. Mun-Bryce. The study was supported by grants from the National Institutes of Health (ROI-NS-21169–10) to G. A. Rosenberg and the Minority Biomedical Research Support Program (GM-08139) at the University of New Mexico.

Address for reprint requests: S. Mun-Bryce, Dept. of Neurology, Univ. of New Mexico, Albuquerque, NM 87131.

Received May 1997; accepted in final form 7 Jan 1998.

REFERENCES


