Role of tumor necrosis factor–α and matrix metalloproteinase–9 in blood-brain barrier disruption after peripheral thermal injury in rats

Laboratory investigation

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Object. A relationship has been found between peripheral thermal injury and cerebral complications leading to injury and death. In the present study, the authors examined whether tumor necrosis factor–α (TNF-α) and matrix metalloproteinase–9 (MMP-9) play a causative role in blood-brain barrier (BBB) disruption after peripheral thermal injury.

Methods. Thirty-two male Sprague-Dawley rats were subjected to thermal injury. One hour later, 8 rats were injected with TNF-α neutralizing antibody, and 8 were injected with doxycycline, an inhibitor of the MMP family proteins; 16 rats did not receive any treatment. Brain tissue samples obtained 7 hours after injury in the treated animals were examined for BBB function by using fluorescein isothiocyanate–dextran and by assessing parenchymal water content. Protein expression of basement membrane components (collagen IV, laminin, and fibronectin) was quantified on Western blot analysis, and MMP-9 protein expression and enzyme activity were determined using Western blot and gelatin zymography. Thermally injured rats that did not receive treatment were killed at 3, 7, or 24 hours after injury and tested for BBB functioning at each time point. Histological analysis for basement membrane proteins was also conducted in untreated rats killed at 7 hours after injury. Results of testing in injured rats were compared with those obtained in a control group of rats that did not undergo thermal injury.

Results. At 7 hours after thermal injury, a significant increase in the fluorescein isothiocyanate–dextran and water content of the brain was found (p < 0.05), but BBB dysfunction was significantly decreased in the rats that received TNF-α antibody or doxycycline (p < 0.05). In addition, the components of the basal lamina were significantly decreased at 7 hours after thermal injury (p < 0.01), and there were significant increases in MMP-9 protein expression and enzyme activity (p < 0.05). The basal lamina damage was reversed by inhibition of TNF-α and MMP-9, and the increase in MMP-9 protein was reduced in the presence of doxycycline (p < 0.05). The authors found that MMP-9 enzyme activity was significantly increased after thermal injury (p < 0.01) but decreased in the presence of either TNF-α antibody or doxycycline (p < 0.01).

Conclusions. The dual, inhibitory activity of both TNF-α and MMP-9 in brain injury suggests that a TNF-α and MMP-9 cascade may play a key role in BBB disruption. These results offer a better understanding of the pathophysiology of burn injuries, which may open new avenues for burn treatment beyond the level of current therapies.

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Key Words • basal laminar protein • burn injury • doxycycline • extracellular matrix • tumor necrosis factor–α neutralizing antibody

Burn encephalopathy is a condition in which neurological complications and sequelae develop in burn victims after thermal injury. Generalized encephalopathy may occur even if the thermal injury is confined to the periphery of the body, and can lead to symptoms including hallucinations, obtundation, cortical blindness, and seizures.4 In 1832, Dupuytren first described “intense cerebral congestion” in autopsies of burn victims.38 In 1928, Kruse35 reported on a 14-month-old baby with extensive second-degree burns in whom mental deterioration, hydrocephalus, and blindness developed. In subsequent decades, hundreds of cases have been reported related to the neurological complications and sequelae of burn injuries.

Thermal injury remains a leading cause of childhood death in the US (according to the Children's Burn Awareness Program in Chicago, 1,000,000 children are...
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injured and 3000 die each year as a result of burn trauma) with
generalized encephalopathy being the most common
neurological complication (14% incidence). Importantly,
current evidence obtained in US soldiers in Iraq who sus-
tained major flash burn injuries indicates that within 24
to 48 hours of injury in some cases, CT-documented brain
edema develops and mental status becomes depressed,
progressing to brain death (personal communication,
Major Gerald Grant, US Air Force, and our unpublished
data). Because they wear protective headgear, affected
soldiers’ heads typically are not directly injured; most of
the damage is inflicted to the trunk and extremities.

Increased vascular permeability develops after pe-
ripheral thermal injury and has been linked to severe ce-
rebral edema, which may lead to neurological complica-
tions that are highly correlated with death.32 In normal
homeostasis, the BBB regulates the passage of exudates
from the vasculature into the brain parenchyma. How-
ever, insults such as ischemia, hemorrhage, or thermal
injury result in upregulation of MMPs that contribute to
remodeling of the ECM at the BBB by breaking down
the major basal lamina components, including collagen
IV, laminin, and fibronectin.27,44 When the ECM is com-
promised, it can no longer regulate the passage of cere-
bral exudates, which leads to generalized cerebral edema.
In our previous study we showed that MMP-9 levels are
specifically increased in the setting of BBB dysfunction
and subsequent edema after thermal injury.52 A causal
relationship between MMPs and BBB disruption after a
peripheral thermal injury remains unproven, however.
Through administration of doxycycline, an MMP inhibi-
tor, and quantification of BBB functioning and basal lam-
a in protein alterations, we hoped to elucidate the caus-
itive role of MMP-9 activity in increased cerebrovascular
permeability after thermal injury.

The authors of previous studies have documented a
relationship between cytokines and MMP expression. Tu-
nor necrosis factor-α is a major deleterious cytokine that
is upregulated in the setting of brain injuries such as stroke
and trauma. Cerebral ischemia causes the upregulation
of TNF-α and TNF-α receptors in neurons, astroglia, and
leukocytes,5,11,26,50 as well as endothelial cells.5,50 A major
site of action for TNF-α is in the microvasculature where
alterations in cytokine expression, adhesion molecule
expression, and permeability are produced.59,90 Within
the cerebral microvasculature, TNF-α increases perme-
ability.1,34,40,42 In our previous study, we demonstrated
early TNF-α upregulation in the brain and serum after severe
peripheral thermal injury.56 These inflammatory
reactions are associated with BBB disruption. The goal of
the present study was to determine whether inhibition of
TNF-α with a neutralizing antibody would reduce BBB
dysfunction after thermal injury. We further investigated
whether TNF-α inhibition reduces BBB disruption via
suppression of MMP-9 activity.

Methods

Animal Preparation

Forty male Sprague-Dawley rats (weight 260–280

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g, Charles River Laboratory) were used in this study.
Throughout the experiment, the animals were housed in
the same care facility with food and water available ad
libitum during a 12-hour light/dark cycle. Animal care
was performed in accordance with guidelines approved
by the National Institutes of Health and the Animal In-
vestigation Committee of the University of Texas Health
Science Center.

The rats were divided into control (8 rats) and ther-
mal injury groups (32 rats). Animals in the thermal injury
group were treated with either TNF-α antibody (8 rats)
or doxycycline (8 rats) or received no treatment (16 rats).
Thermally injured rats that did not receive treatment were
killed at 3, 7, or 24 hours after injury. Samples obtained
in these rats were tested for BBB breakdown with FITC-
dextran and spectrophotometry. Basal lamina protein
expression analysis and measurement of MMP-9 protein
levels and enzyme activity were also performed. Rats in
the treatment groups were only tested at 7 hours after
thermal injury because this corresponds to the time of
maximum activity of MMP-9.52

Peripheral Thermal Injury

The animals were subjected to thermal injury based on
previously described procedures.50 Briefly, the rats
were anesthetized with a face mask using 2% halothane
in 30% O2, and then submerged horizontally for 6 seconds,
supine, in either 100°C water for the thermal injury group
or 37.5°C for the control group. The head, hindlimbs, a
portion of the abdomen, and the genitalia were held out of
the water. In the thermal group, this procedure produced
a third degree burn that affected 60–70% of the animals’
body surface area.52 The animals were given analgesic
relief throughout the entire procedure, and body tempera-
ture and mean arterial blood pressure were monitored.

Inhibition of TNF-α and MMP-9

Endogenous TNF-α expression, which is induced by
peripheral thermal injury, was blocked by administration
of neutralizing TNF-α antibody postinjury. For this pur-
pose, polyclonal rabbit anti–mouse TNF-α neutralizing
antibody (0.30 mg/kg; Genzyme) dissolved in nonpyro-
genic sterile saline was injected intravenously 1 hour af-
after injury. For inhibition of MMP-9, doxycycline (Sigma)
dissolved in saline was administered intraperitoneally 1
hour after injury. We used a doxycycline dose of 10 mg/
kg, which was shown to effectively reduce MMP-9 levels
in rats in our preliminary study.

Identification of BBB Function

Dextran labeled with FITC was used to determine the
extent of BBB disruption and permeability alterations at
3, 7, and 24 hours after thermal injury (in 4 untreated rats
killed at each time period) and 7 hours after injury in the
rats that received TNF-α antibody or doxycycline. Blood-
brain barrier breakdown allows water and blood-borne
substances to pass into the brain parenchyma, leading to
vasogenic brain edema. Fluorescein isothiocyanate–dex-
tran (average molecular weight 40,000 daltons) is com-
monly used to measure vascular permeability because it
remains in the brain capillary lumen with no tracer leakage around the vessels in vivo. Rats were injected with 0.25 ml of 5% FITC-dextran per 100 g of body weight via the femoral artery 5-10 minutes prior to planned death.

Cryostat brain sections were collected in 10-μm-thick slices. Images were obtained at 400 × original magnification for analysis of FITC-dextran leakage from cerebral vessels using an image analysis system (AxioVision 4.5TM). For spectrophotometric analysis, the brain tissue was thoroughly flushed with 0.9% saline to eliminate vascular stasis of FITC-dextran. The right hemispheres were then weighed and placed in 6 ml of a 50% trichloroacetic acid solution. The samples were homogenized and centrifuged for 45 minutes at 4000 rpm to form a pellet. The aqueous upper phase was analyzed using a Beckman Coulter AD 340 plate reader. A standard curve for control was generated using an FITC-dextran serial dilution. The magnitude of BBB disruption was then identified by measuring the extent of tracer leakage.

Brain edema was measured directly by assessing the water content in the brains of thermally injured rats with and without treatment. Rat brains were harvested and immediately weighed after extraction to determine their wet weight before being dried in an oven at 70°C for 72 hours and weighed again to obtain the dry weight. The formula (wet weight – dry weight)/wet weight × 100 was used to calculate the water content and expressed as a percentage of the wet weight.

**Basal Lamina Protein Expression Determined by Western Blot Analysis**

- Protein expression of basement membrane components, including collagen IV, laminin, and fibronectin was quantified with Western blot analysis in thermally injured and control animals. Supernatants were used as whole-tissue lysates and the protein concentration was determined with the Bradford assay (Bio-Rad). Equal amounts of protein (30 μg/well) were separated on 10% sodium dodecyl sulfate–polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Bio-Rad). The membranes were blocked for 1 hour at room temperature with 5% skim milk in Tris-buffered saline with Tween 20, and then incubated with the primary antibodies, a polyclonal rabbit anti-rat laminin antibody (1:500, R&D Systems), a polyclonal goat anti-collagen IV antibody (1:500; SouthernBiotech) or a polyclonal rabbit anti-rat fibronectin antibody (1:500; Sigma) overnight at 4°C. After incubation with the secondary antibodies for 1 hour at room temperature, detection of immunoreactive bands was performed with the ECL Western blotting system (GE healthcare). Equal protein loading was confirmed with intracellular protein β-actin (goat polyclonal anti-β-actin antibody, dilution 1:1000; Santa Cruz Biotechnology). The intensity of protein expression was quantified using the ChemiGeniusQ Imaging Analysis System.

**Matrix Metalloproteinase–9 Expression**

Western blot analysis was used to detect protein synthesis of MMP-9 quantitatively in thermally injured rats with and without treatment. Primary monoclonal anti-rat MMP-9 antibody (VII C2, 1:200, Oncogene Science) was incubated with the membrane for 1 hour at 25°C. Similarly prepared samples as used in Western blot analysis (equal volumes of 10 μl) were used for gelatin zymography to quantify MMP-9 enzyme activity in thermally injured rats with and without treatment. Samples were processed as previously described.

Because the actual levels of active versus proforms cannot be reliably quantified, pro-MMP and active bands were combined to obtain total MMP levels for quantitative analysis. Authors of some studies have suggested that dynamic processing of active MMPs occurs at the cell surface. Relative total levels of MMP protein expression (both pro- and active MMP) and relative total gelatinolytic activity (pro- and active MMP) were quantified and presented as fold increases in comparison with controls, using an image analysis program (ChemiGeniusQ System).

**Statistical Analysis**

All data are expressed as means ± standard errors. Statistical analysis was performed with commercially available software (SPSS for Windows, version 15.0). The differences among multiple groups were assessed using the 1-way ANOVA with statistical significance set at p < 0.05. Post hoc comparison between groups was further undertaken with the least significant difference method. Overall MMP-9 protein and enzyme activity levels were determined in the presence of TNF-α antibody and MMP-9 inhibitor after thermal injury and correlated with cerebral vascular damage as measured by FITC-dextran extravasations, brain edema, and basal laminar protein loss.

**Results**

**Blood-Brain Barrier Disruption After Skin Burn**

In the present study, we examined the leakage of FITC-dextran from cerebral microvessels into the brain parenchyma in uninjured control rats and in thermally injured rats killed 3, 7, and 24 hours postinjury (4 rats each), and compared these findings with the results obtained in the thermally injured rats killed 7 hours after injury that had been treated with TNF-α antibody or doxycycline. Leakage was observed on fluorescence microscopy (Fig. 1) and quantified with spectrophotometry (Fig. 2). An intact BBB prevents extravasation of FITC-dextran from the microvasculature; therefore, no tracer was observed surrounding the vessels in the control rats (Fig. 1A). An insufficient BBB allows leakage of the tracer from the vessels into the surrounding tissue and is seen at 7 hours after thermal injury (Fig. 1B). Decreased parenchymal tracer content was observed surrounding the vessels in rats that received TNF-α antibody or doxycycline (Fig. 1E and F). These observations are corroborated with the spectrophotometrically quantified amounts of FITC-dextran in the brain tissue in rats in each experimental group (Fig. 2). The 1-way ANOVA analysis indicated a significant increase in FITC-dextran leakage into surrounding brain tissue in the thermally injured rats compared with controls; the leakage peaked at 7 hours after injury (F1,16 = 4.9; p < 0.05). Treated rats showed a significant decrease
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**Fig. 1.** Fluorescence microscopy photomicrographs showing FITC-dextran staining of right cerebral hemisphere tissue from an uninjured control rat (A), and in rats 7 hours after thermal injury, untreated (B), treated with TNF-α neutralizing antibody (C), and treated with doxycycline (D). Blood vessels are indicated with thin arrows, and in the untreated, injured rat tissue, thick arrows indicate the increase in fluorescent labeling in the surrounding parenchyma due to tracer leakage. The intensity of fluorescence was reduced to baseline levels in the injured animals that received either treatment.

in BBB insufficiency at 7 hours compared with untreated injured animals ($F_{2,12} = 7.5; p < 0.01$).

Brain edema was further assessed as a percentage change in the brain’s water content 7 hours after thermal injury in rats with or without TNF-α or MMP-9 inhibition (Fig. 3). The 1-way ANOVA revealed a significant difference ($F_{3,16} = 10.7, p < 0.01$) in the percentage of brain water content among the groups (control, thermal injury without treatment, and thermally injured rats that received TNF-α antibody or doxycycline). The post hoc analysis further indicated that thermal injury significantly increased the water content in the brain ($p < 0.01$) and that treatment with either TNF-α or doxycycline significantly reduced brain edema in thermally injured rats ($p < 0.01$). No difference was detected in brain water content between uninjured control rats and the thermally injured rats that received either treatment.

**Basal Lamina Protein Loss After Thermal Injury**

The extent of damage to the major components of the BBB microvascular basal lamina (collagen IV, fibronectin, and laminin) caused by thermal injury was measured on Western blot analysis (Fig. 4). The 1-way ANOVA analysis indicated significant decreases in the levels of each basal laminar protein at 7 hours after thermal injury (collagen IV, $F_{3,16} = 51.8$; fibronectin, $F_{3,16} = 32.3$; and laminin, $F_{3,16} = 16.1$; $p < 0.01$). This reduction in protein levels was restored to control levels in rats that received doxycycline. Although restoration of protein levels was not complete in animals that received TNF-α inhibition, protein levels were significantly greater than in untreated thermally injured rats ($p < 0.05$).

**Matrix Metalloproteinase–9 Upregulation After Injury**

Matrix metalloproteinase–9 protein expression and activity were quantified using Western blot analysis and zymography to determine the effects of TNF-α upregulation on MMP-9–associated brain damage. This was performed in 4 control rats, 4 rats treated with TNF-α antibody, 4 rats that received doxycycline, and 4 injured rats that did not receive treatment (killed at 7 hours after injury). The ANOVA analysis demonstrated that, although mild, the skin burn caused a significant increase in MMP-9 protein expression ($F_{3,16} = 7.5, p < 0.01$), shown by a relative image density of $1.4 \pm 0.1$ compared to the control group (arbitrarily assigned a reference value of $1.0 \pm 0.0$; Fig. 5A). Although a minimal change in MMP levels was observed in the rats treated with TNF-α antibody, doxycycline treatment was found to generate a significant decrease in MMP-9 protein levels compared with those measured 7 hours after thermal injury in untreated rats ($p < 0.01$). Gelatin zymography further demonstrated a significant ($F_{3,16} = 23.4; p < 0.01$) increase in MMP-9 activity in thermally injured rats, shown by a relative image density of $19.6 \pm 2.8$, compared with the uninjured control rats (arbitrarily assigned as $1.0 \pm 0.0$; Fig. 5B). This increase was significantly reduced in injured rats with TNF-α inhibition, as shown by image density values of $3.5 \pm 0.6$ ($p < 0.01$). Furthermore, as predicted, a significant reduction in MMP-9 protein and activity was seen in thermally injured animals after inhibition of MMP-9 with doxycycline ($p < 0.01$). This finding was illustrated by the image density values of $1.1 \pm 0.1$ for Western blot analysis and $2.3 \pm 0.2$ for zymography, in association with the observed reversal of BBB dysfunction and basal lamina loss.
**Discussion**

In the present study, we demonstrated that peripheral thermal injury causes BBB damage, as demonstrated by increased BBB permeability and brain edema. This damage is associated with increased MMP-9 expression and a decrease in basilar laminin proteins (collagen IV, fibronectin, and laminin), which was ameliorated by inhibition of either TNF-α or MMP-9. Furthermore, TNF-α neutralizing antibody suppresses MMP-9 activity and reverses the basilar laminin protein loss caused by peripheral thermal injury, while also improving BBB integrity. These results illustrate the role of a TNF-MMP cascade in regulating BBB integrity after thermal injury.

**Blood-Brain Barrier Disruption Caused by MMP Expression**

The BBB exists between the systemic circulatory system and the cerebral parenchyma, and is comprised of interendothelial tight junctions, the basilar lamina, and perivascular astrocytes. When this barrier is compromised, vascular extrudes leak into the surrounding tissues, causing swelling in the cranial cavity. The basilar lamina of the ECM in the BBB surrounds and anchors endothelial cells and astrocytes to provide a structural barrier to extravasation of blood elements. When toxic proteases are upregulated due to traumatic conditions, the balance of basilar lamina proteins becomes tipped in favor of degradation, which causes cerebral edema, hemorrhage, and cell death. Our study indicates that loss of basilar laminin protein was caused by peripheral thermal injury and is associated with BBB dysfunction.

Matrix metalloproteinases are directly involved in tissue remodeling during development and homeostasis, but are also produced by endothelial cells, microglia, and astrocytes in response to pathological conditions such as atherosclerosis, arthritis, cancer, and neurodegeneration. Collagen IV, laminin, and fibronectin are the target proteins degraded by MMPs. The authors of recent studies have emphasized the role of MMPs in regulating the integrity of the BBB after injury. In the central nervous system, the early appearance of activated MMP-9 is associated with alterations in BBB permeability, the formation of vasogenic edema after cerebral ischemia, and other acute cerebral injuries. In addition, pharmacological inhibition of MMPs can reduce edema after cerebral ischemia. Also, MMP-9-deficient knockout mice have been shown to have reduced BBB disruption and edema after cerebral ischemia and traumatic brain injury. Finally, our previous data show an increase in cerebral expression of MMPs, especially MMP-9, at the level of transcription (mRNA), translation (protein), and
enzyme activity, and demonstrates a temporal association between BBB breakdown and cerebral expression of MMPs in thermally injured rats. Our results in the present study confirm the results of previous work suggesting a correlation between disruption of neurovascular integrity and MMP-9 expression after thermal injury.

In the present study, doxycycline, a member of the tetracycline family, was used to directly inhibit MMP-9 after thermal injury. It has been speculated that the inhibitory effects of doxycycline on MMPs are due to reduced MMP mRNA stability and MMP synthesis. In addition, doxycycline is well recognized for its therapeutic efficacy in treating MMP-mediated disease. Therefore, in blocking MMP-9 activity with doxycycline, our results further suggest a cause-and-effect correlation between BBB disruption and MMP-9 expression after thermal injury. Although doxycycline may have diverse systemic effects and its role in MMP regulation involves other mediators such as the tissue inhibitors of metalloproteinase-1 (TIMP-1), the reversal of BBB disruption by doxycycline is likely to involve MMP activity because reduced MMP-9 expression was associated with this reversal.

In addition, although brain MMP-9 mRNA is greatly increased by 3 hours after thermal injury and is followed by an elevation in MMP-9 protein levels and enzyme activity through 72 hours, serum MMP-9 protein and enzyme activity levels remain low with only small increases in

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Fig. 4. Graphs and Western blot analysis showing the density of the major components of the BBB microvascular lamina at 7 hours after injury. A: A significant decrease in the levels of collagen IV was seen (F<sub>3,16</sub> = 51.8; p < 0.01). Decreased protein levels of collagen IV were significantly reversed in rats treated with TNF-α or doxycycline (#p < 0.05). B: Fibronectin damage was significant (F<sub>3,16</sub> = 16.1; p < 0.01). Treatment with either TNF-α antibody or doxycycline significantly reversed the decrease in fibronectin levels caused by thermal injury (#p < 0.05). C: Laminin levels were significantly decreased (F<sub>3,16</sub> = 16.1; p < 0.01). The decrease was significantly reversed with doxycycline treatment, and some reversal was seen after TNF-α antibody treatment (#p < 0.05).
activity at 7 hours postinjury. This finding suggests that early cerebral expression of MMP for up to 7 hours after thermal injury is not caused by systemic MMP expression. This finding indicates that other traumatic mediators, such as cytokines, may act as triggers to induce cerebral MMP expression and associated BBB dysfunction.

**Detrimental Role of Inflammatory Reaction After Thermal Injury**

Tumor necrosis factor-α is a proinflammatory cytokine released in response to tissue damage such as in stroke and brain trauma. Increases in circulating levels of TNF-α are found in diseases such as bacterial meningitis, AIDS-related dementia, multiple sclerosis, and septicemia. A major site of action for TNF-α is the microvasculature, where alterations in cytokine expression, adhesion molecule expression, and permeability are produced. Within the cerebral microvasculature, TNF-α increases permeability. The early appearance of inflammatory cytokines in the systemic circulation, including TNF-α, interleukin-1, and interleukin-6, was demonstrated following thermal injury both in humans and animals. Upregulation of cytokines correlates proportionally with the extent of the burn injury.

Our previous study demonstrated a significant increase of TNF-α and interleukin-1β as well as intercellular adhesion molecule-1 (ICAM-1) in the brain and serum in the same peripheral thermal injury model we describe in the present study. Although systemic anti-TNF-α therapy in the setting of systemic trauma such as burn injuries probably influences a myriad of effects related to inflammation and injury responses, in the present study, we found evidence that a blockade of TNF-α activity reduces BBB dysfunction and MMP-9 expression. This finding suggests that the increase in TNF-α-mediated systemic inflammatory reactions following thermal injury play a causal role in inducing BBB disorder and MMP-9 expression. The systemic TNF-α upregulation might stimulate cerebrovascular reactions and induce brain damage in skin burn injuries by activating MMP-9.

Clearly a wide variety of systemic inflammatory responses are induced after extensive burns, and changes occur in blood pressure, hormones, circulating leukocytes, viscosity, and oxygenation, among other factors. Tumor necrosis factor-α and other cytokines are almost certainly involved, and whatever disrupts the BBB based on any etiology probably involves MMP activation in the final process. The results of this study may help to establish a direct relationship between thermal injury and TNF-mediated MMP activation as part of the pathogenesis of BBB disruption. Previously, an associated increase in mRNA levels of MMP-8, -9, -12, and TNF-α, as well as in their protein synthesis, has been reported in delayed-type hypersensitivity lesions in the brain. The authors of a recent study demonstrated a causal relationship between TNF-α and MMP expression after cerebral ischemia, in association with cerebral integrity disorders. Treatment with anti-TNF-α neutralizing antibody ameliorated brain infarction and edema by reducing MMP upregulation in ischemic brain tissue. In vitro studies, activation of brain microvascular endothelium with the proinflam-
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matory cytokines TNF-α and interleukin-1β resulted in selective upregulation of MMPs such as MMP-1,-2,-3,-7,-9, and -12.23,25 The addition of dexamethasone, a chemotherapy drug, partially inhibited the cytokine-induced upregulation of MMP-9.25

Conclusions

Although systemic administration of cytokine or protease inhibitors may affect a number of processes, our findings suggest a direct link between TNF-α and MMP-9 in the degradation of the BBB. Results of sensitive FITC-dextran measurement, brain edema detection, and basal lamina protein analysis suggest that BBB destruction is associated with TNF-α expression and MMP-9 activity. Understanding the mechanisms of the TNF-MMP pathway underlying BBB dysfunction may provide better insight into neurological disorders that develop after thermal injury, leading to improved pharmacological target therapies.

Disclosure

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Disclaimer

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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