Chemokine detection in the cerebral tissue of patients with posttraumatic brain contusions

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Object. The clinical outcome of patients with severe head injuries is still critically dependent on their secondary injuries. Although hypoxia and hypotension appear to mediate a substantial proportion of secondary injuries, many studies associate secondary brain injury with neuroinflammatory responses. Chemokines have been detected in the cerebrospinal fluid but not in the brain tissue of patients with head trauma. This study was performed to determine if chemokines were expressed in pericontusional brain tissue in patients with moderate or severe head trauma who underwent surgical evacuation of their brain contusions.

Methods. Twelve patients with posttraumatic cerebral contusion requiring a surgical evacuation were studied. A 20- to 40-mg sample of white matter was removed from the surgical cavity in the pericontusional area. Two patients undergoing elective surgery for clip ligation of an unruptured aneurysm were used as controls. The median interval from trauma to biopsy procedure was 44 hours (range 3–360 hours). Total RNA was isolated from these samples and a ribonuclease protection assay was performed to measure the mRNA levels of several chemokines: CCL2, CCL3, CCL4, CCL5, CXCL8, CXCL10, and XCL1.

Results. The CCL2, a monocyte chemoattractant produced by activated astrocytes, was the most strongly expressed chemokine, followed by CXCL8, CCL3, and CCL4. The chemokines CXCL10 and CCL5 were expressed at very low levels, and XCL1 was not detected.

Conclusions. Chemokine activation occurs early after moderate or severe head trauma and is maintained for several days after trauma. This event may contribute to neuroinflammatory exacerbation of posttraumatic brain damage in the pericontusional brain tissue. (DOI: 10.3171/JNS/2008/108/5/0958)

KEY WORDS • brain inflammation • brain trauma • cerebral contusion • chemokine • secondary brain injury

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HE clinical outcome of patients with severe head injuries is still critically dependent on the extent of residual brain damage that evolves after the initial insult. Whereas primary brain injury results from mechanical forces applied to the skull and brain at the time of impact, secondary brain injury is the result of the pathological processes initiated by the primary insult. The central goal in the critical care of head-injured patients is to prevent, or treat rapidly, potential secondary injury. Although hypoxia and hypotension appear to mediate a substantial proportion of secondary injuries, many studies associate secondary brain injury with neuroinflammatory responses. Chemokines have been detected in the cerebrospinal fluid but not in the brain tissue of patients with head trauma. This study was performed to determine if chemokines were expressed in pericontusional brain tissue in patients with moderate or severe head trauma who underwent surgical evacuation of their brain contusions.

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Abbreviations used in this paper: CNS = central nervous system; GCS = Glasgow Coma Scale; TBI = traumatic brain injury.
Human chemokines in posttraumatic brain contusions

CC), generally do not act on neutrophils but attract monocytes, eosinophils, basophils, and lymphocytes. The other 2 chemokine families consist of the one designated C (acting primarily on the T resting cells) and the one named CXXXC (acting primarily on the natural killer cells).26

Chemokine receptors are classified into 4 subgroups. These receptor subgroups have been designated as CXCR, CCR, XCR, and CX3CR.43 The identification of chemokine receptors is important from a pathological point of view because they permit the synthesis of antagonist molecules.1

Although chemokine receptors are constitutively expressed by resident CNS cells, the expression of chemokines in brain tissue2,32 occurs at low levels, and their production can be increased by inflammatory mediators. Numerous detailed studies of CNS chemokines and their receptors have been published, and it is now clear that endogenous CNS cells synthesize distinct chemokines and may respond to chemokine stimulation by increasing the expression of chemokine receptors.8

Chemokines have been detected in the cerebrospinal fluid of patients with head trauma,11 but studies of brain tissue are lacking. In this prospective study we measured the expression patterns of chemokines in the pericontusional brain tissue in patients with moderate or severe head trauma.

Clinical Materials and Methods

This study was conducted at the Department of Neurosurgery at the University of Brescia, Spedali Civili, Italy, and was approved by the local ethics committee. Written informed consent was obtained from the patients whenever possible; otherwise, written information was given to their next of kin. Written consent was obtained from all surviving patients as soon as they regained mental competency.

Patient Population

Twelve patients with moderate (GCS Score 9–12) to severe (GCS Score ≤ 8) head trauma and a computed tomography scan of the brain demonstrating features compatible with cerebral contusion were studied. In addition, 2 control patients undergoing clipp ligation of an unruptured cerebral aneurysm were included. All patients underwent a craniotomy, dural opening, and evacuation of the contusion following neurological deterioration associated with a severe and prolonged increase in intracranial pressure and/or an increase in the volume of the cerebral contusion.

Six patients with severe and 6 with moderate head trauma were included in the study. The cause of head trauma was a road accident in 8 cases, a fall in 3 cases, and a work-related accident in 1 case. Ten patients were male and 2 were female, and their mean age was 45.2 ± 21.4 years (mean ± standard deviation). The median admission GCS score was 9 (range 6–13) and the median presurgery GCS score was 9 (range 4–12).

Surgical evacuation with biopsy sampling was performed within the first 24 hours of the injury in 5 patients who exhibited rapidly enlarging cerebral contusions with signs of intracranial hypertension on neurological examination and computed tomography scans of the brain. In the other 7 patients, delayed enlargement of the cerebral contusion occurred, and the craniotomy was performed between 35 hours and 15 days after trauma. Overall, the median interval from trauma to biopsy was 44 hours (range 3–360 hours).

Biopsy Sampling of Cerebral Tissue

After complete resection of the contused cerebral tissue by using a surgical pincer, a sample of white matter (20–40 mg) was removed from the surgical cavity corresponding to the edematous pericontusional area. The sample was washed with physiological saline solution, dried with sterile gauze, placed in a sterile tube, and rapidly frozen in a dedicated freezer.

The 2 control patients had undergone elective surgery for clip ligation of an unruptured aneurysm in the anterior communicating artery region. Following periternal craniotomy, opening of the dura mater and basal cisterns was performed, and a small part of the posterior gyrus rectus was removed to expose the aneurysm sac. A sample of white matter (20–40 mg) was obtained and treated as described for patients with posttraumatic cerebral contusion.

Extraction of RNA From Cerebral Tissue

Total mRNA was isolated from the brain by using the guanidinium-thiocyanate method with a commercially available total RNA isolation reagent (TRIzol, Life Technologies)14 (1 ml/50–100 mg tissue). The homogenized solution was incubated for 5 minutes at 15–30°C to permit complete dissociation of nucleoprotein complexes, followed by addition of 0.2 ml of chloroform in 1 ml of TRIzol. Samples were then centrifuged, the aqueous phase was recovered, and RNA was precipitated with isopropyl alcohol by centrifugation at 10,000 G for 10 minutes at 4°C. The RNA precipitate was washed with 75% ethanol and air-dried before resuspension in ribonuclease-free water. The solution was passed a few times through a pipette tip and incubated for 10 minutes at 55°C.

Ribonuclease Protection Assay

Chemokine mRNAs were detected using the RiboQuant Multi-Probe RPA kit (template sets hCK-5, Pharmingen) according to the instructions of the supplier. Riboprobes were labeled with phosphorus-32 and hybridized overnight with 5 μg of RNA. The hybridized RNA was treated with ribonuclease and purified according to the RiboQuant protocol. Protected RNAs were then separated on a 5% denaturing polyacrylamide gel. The gel was transferred to filter paper, dried under a vacuum, and exposed using both a phosphoimager and x-ray film (XAR film, Eastman Kodak Co.) with intensifying screens at −70°C. Results were evaluated using densitometric analysis. Values were expressed as the percentage of housekeeping gene (L32) expression. This kit permitted the analysis of the following chemokines (alternate names appear in parentheses after each chemokine): CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CXCL8 (IL-8), CXCL10 (IP-10), and XCL1 (SCM-1α).

Data Presentation

We expressed continuous variables as the mean ± stan-
standard deviation or as the median and range, and discrete variables were expressed as counts and the percentage, unless otherwise stated.

**Results**

In all 12 cases analyzed, mRNA levels for different chemokines were detectable by using a ribonuclease protection assay (Fig. 1). There were no detectable levels of chemokine mRNA in either of the 2 control samples (data not shown).

The chemokine CCL2 was the one most consistently and strongly expressed, followed by CXCL8, CCL3, and CCL4, which were expressed at intermediate levels; CXCL10 and CCL5 were expressed at very low levels; and XCL1 was not detected. Expression of CCL2 was elevated as early as 3.5 hours after trauma, reached a first peak at 14 hours after trauma, and a second peak at 8 days (Fig. 2). The CXCL8 expression reached a single peak at 24 hours, and mRNAs for CCL2 and CXCL8 were both detectable in pericontusional tissue as early as 3 hours after trauma.

**Discussion**

In this study we have characterized the chemokine expression pattern in the brain parenchyma of patients with traumatic head injury. The CCL2 appeared to be the mostly highly expressed chemokine in the damaged human brain tissue; CCL2 (also known as MCP-1) has been reported to be an important chemokine produced by activated astrocytes in rodent models of TBI and autoimmune encephalomyelitis. Glabinski et al. postulated that this cytokine is probably important in triggering the immune response to a nonimmune injury in the brain through astrocyte activation and subsequent attraction of mononuclear phagocytic cells. In fact, they discovered an early CCL2 mRNA expression by astrocytes in a rat model of penetrating TBI. In our study, although we cannot describe the source of CCL2, we noted that this chemokine was highly expressed in the pericontusional area (at least 3 times more than the other chemokines), and we found a high expression of CCL2 also in those patients who underwent early surgery (Fig. 1). However, the cellular source of CCL2 is still unknown.

The chemokine CXCL8 (also known as IL-8) was highly expressed in the traumatized human brain. The sources of the CXCL8 are likely to be microglia, endothelial cells, monocytes, and neutrophils. It is interesting to note that the CXCL8 levels have been correlated with increased blood–brain barrier permeability. The chemokine CXCL8 is considered to be an essential stimulus for neutrophil chemotaxis. Whalen et al. have demonstrated the high expression of CXCL8 in the cerebrospinal fluid of children with TBI. The worst outcome occurred in patients with the

![Bar graph](image-url)
highest expression of CXCL8; this chemokine induces neutrophil-dependent microvascular damage in a variety of in vitro and in vivo inflammatory models. However, no data were available at the time of the study regarding CNS inflammation, especially of the human brain, after traumatic head injury.

Our results suggest that some differences may exist between CXCL8 and CCL2 expression. In particular, CXCL8 expression reached a peak 24 hours after head trauma, whereas CCL2 expression was characterized by a first peak at 14 hours. We speculate that CCL2 is an early expressed chemokine that is probably derived from a noninflammatory activation of the astrocytes, whereas CXCL8 is a late mediator that initiates its activity when the brain inflammation has been established, acting mainly on neutrophil recruitment.

The role of neutrophils in triggering neuroinflammation is well established; however, the role of lymphocyte activation in acute CNS inflammation is still unclear. Neutrophils are believed to be early actors in the CNS immune response, and this hypothesis is supported by our data. We detected very low or no mRNA expression of CXCL10 and XCL1, which are 2 chemokines that have important effects on lymphocyte trafficking in TBI; on the contrary, the 2 most expressed chemokines are CCL2 and CXCL8, which act mainly on neutrophil recruitment.

The time course analysis on which our hypothesis is based has a couple of drawbacks. First, serial specimens were not obtained in the same patient. Second, we assumed that the initial TBI was the only critical event; however, the delayed contusion enlargement may have played a comparable role.

**Conclusions**

The CCL2 and CXCL8 chemokines are strongly expressed in the brain tissue of patients with posttraumatic brain contusions. Neuroinflammation contributes to secondary ischemic brain damage and to contusion enlargement; therefore CCL2 and CXCL8 might play a relevant pathogenetic role. Future studies should better characterize the kinetic and cellular sources of chemokines in TBI.

**References**

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