TAURINE IMPROVES FUNCTIONAL AND HISTOLOGICAL OUTCOMES AND REDUCES INFLAMMATION IN TRAUMATIC BRAIN INJURY

Y. SU,1,2 W. FAN,3,4 Z. MA,1,5 X. WEN,1,5 W. WANG,1,5 Q. WU5 AND H. HUANG1,2

1 The Graduate School, Tianjin Medical University, Tianjin 300070, PR China
2 Tianjin Neurosurgical Institute, Tianjin Huanhu Hospital, Tianjin 300060, PR China
3 Baoding NO. 1 Hospital, Baoding, Hebei 071000, PR China
4 Abbreviations: BCA, biocinchoninic acid; CBF, cerebral blood flow; CHI, close head injury; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; G-CSF, Granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon-gamma; IL, interleukin; MCP-1, monocyte chemotactic protein-1; mFPI, moderate fluid percussion injury; mNSS, Modified Neurological Severity Score; PBS, phosphate-buffered solution; RANTES, regulated on activation, normal T cell expressed and secreted; SCI, spinal cord injury; Tau, taurine; Tau-CL, taurine chloramine; TBI, traumatic brain injury; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor.

E-mail address: huanghuling@126.com (H. Huang).

Key words: taurine, traumatic brain injury, cytokine, inflammation, Luminex.

Abstract—We investigated the effect of taurine on inflammatory cytokine expression, on astrocyte activity and cerebral edema and functional outcomes, following traumatic brain injury (TBI) in rats. 72 rats were randomly divided into sham, TBI and Taurine groups. Rats subjected to moderate lateral fluid percussion injury were injected intravenously with taurine (200 mg/kg) or saline immediately after injury or daily for 7 days. Functional outcome was evaluated using Modified Neurological Severity Score (mNSS). Gliarial fibrillary acidic protein (GFAP) of the brain was measured using immunofluorescence. Concentration of 23 cytokines and chemokines in the injured cortex at 1 and 7 days after TBI was assessed by Luminex xMAP technology. The results showed that taurine significantly improved functional recovery except 1 day, reduced accumulation of GFAP and water content in the penumbral region at 7 days after TBI. Compared with the TBI group, taurine significantly suppressed growth-related oncogene (GRO/KC) and interleukin (IL)-1β levels while elevating the levels of regulated on activation, normal T cell expressed and secreted (RANTES) at 1 day. And taurine markedly decreased the level of 17 cytokine: eotaxin, Granulocyte colony-stimulating factor (G-CSF), Granulocyte-macrophage colony-stimulating factor (GM-CSF), Interferon-gamma (IFN-γ), IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17, leptin, monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), and only increased the level of MIP-1α in a week. The results suggest that taurine effectively mitigates the severity of brain damage in TBI by attenuating the increase of astrocyte activity and edema as well as pro-inflammatory cytokines. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

INTRODUCTION

Traumatic brain injury (TBI) is a major cause of death and disability. It is associated with a complex sequence of inflammatory responses characterized by glial activation, neutrophil and macrophage recruitment, upregulation of adhesion molecules, and secretion of cytokines (Werner and Engelhard, 2007; Lotocki et al., 2009). Brain injury shows the pathophysiology of inflammation including diffuse brain edema and neurological functional deficits. In addition, TBI elicits reactive astrogliosis reflecting the degree of brain injury (Laird et al., 2008).

Clinical and experimental TBI is associated with altered systemic and brain levels of cytokines including interleukin (IL)-1, IL-6, IL-10, and tumor necrosis factor-alpha (TNF-α) (Morganti-Kossman et al., 1997; Maier et al., 2001; Ziebell and Morganti-Kossmann, 2010). Recently, a novel Luminex assay was used to detect 23 cytokines in the cerebral cortex of rats at 3 h and 24 h following a moderate fluid percussion injury (mFPI). The results indicated mFPI significantly elevated levels of IL-1α, IL-6, growth-related oncogene (GRO/KC, systemic name: CXCL1); Macrophage inflammatory protein-1α (MIP-1α), and TNF-α in the cortex, but decreased levels of IL-4, IL-12, IL-13, IL-17 and IL-18 (Redell et al., 2013). Luminex is currently the most widely used multiplex biomarker analysis technology with distinct advantages of higher throughput, and smaller sample volume. It consumes less time and is associated with lower cost compared with enzyme-linked immunosorbent assay (ELISA). This method enables a more complete assessment of the cytokine cascade that occurs following TBI. It has also been used for the detection of cytokines in the cerebrospinal fluid (CSF) and serum of patients after TBI (Buttram et al., 2007; Hergenroeder et al., 2010).

Taurine (Tau), 2-aminoethane sulfonic acid, acts as an osmoregulator (Schaffer et al., 2000), neuromodulator, calcium regulator (El Idrissi, 2008), antioxidant (Messina and Dawson, 2000), and provides neuroprotection against excitotoxic cell death (Huxtable, 1989). It has been associated with potent anti-
inflammatory effects in a variety of models of systemic inflammation, including the spinal cord injury (SCI) (Nakajima et al., 2010), ischemic stroke (Sun et al., 2012), hepatic ischemia reperfusion (Zhang et al., 2010), and lung injury (Abdih et al., 2000). Specifically, taurine has been shown to diminish the production of cytokines, such as IL-1β, IL-6 and TNF-α in SCI and risk of ischemic stroke (Nakajima et al., 2010; Sun et al., 2012). Therefore, we hypothesized that taurine may attenuate TBI-induced inflammatory response.

Our aim was to investigate the interaction of taurine with the cascade of inflammation in a rat model of TBI using Luminex technology. We also examined the impact of taurine in cerebral edema, astrocyte activity and neurological function after TBI.

**EXPERIMENTAL PROCEDURES**

**Animals**

Adult male Sprague–Dawley rats, weighing between 260 and 300 g and obtained from the Experimental Animal Laboratories of the Academy of Military Medical Sciences (Beijing, China) were used for all experiments. Animal care was in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, the National Academy of Sciences, Washington, DC, USA). Rats were housed under standard conditions of temperature (23 ± 2 °C) and light (12-h light/dark cycle), and were provided with food and water ad libitum during the experiment.

**Experimental groups**

In this study, 72 rats were randomized into three groups (n = 24 in each group): (1) sham group: rats were subjected to identical surgical procedures except for brain injury, and administered saline only; (2) TBI group: rats were subjected to FPI and received saline only; and (3) Tau group: TBI rats were administered taurine (200 mg/kg/day, Sigma, St. Louis, MO, USA) by tail intravenous injection, once daily for either 1 or 7 days after TBI. The three groups were then divided into two subgroups (n = 12 for each time-point in each group) at 1 and 7 days post-injury, respectively. All rats were sacrificed 1 and 7 days after TBI for further analysis. The number of animals used in each group was present in Tables 1 and 2.

**FPI model**

TBI model was modified from previous study (Kelley et al., 2006). Rats were intraperitoneally anesthetized with 10% chloral hydrate (10 μl/100 g), and then immobilized in a stereotaxic frame. After exposing the skull, a 5.0-mm craniotomy was performed over the left parietal bone, 4.5 mm posterior from bregma and 2.5 mm lateral to the sagittal suture, ensuring the integrity of the dura. A female Luer-Lock fitting was then cemented to the skull with cranioplastic cement. The rats were connected to the fluid percussion device (University of Virginia, USA) via the Luer–Lock fitting, and an overpressure of 1.8 atm was delivered causing a moderate brain injury. Body temperature was monitored by a rectal probe, maintaining at 37 ± 1 °C with a heating pad during the experiment. After surgery, the skin was closed with staples and rats were allowed to recover from anesthesia.

**Neurological function evaluation**

The Modified Neurological Severity Score (mNSS), previously described by Chen et al., was used to evaluate post-injury functional impairment (Chen et al., 2001). As a composite of the motor, sensory and reflex tests, the mNSS was graded on a scale of 0–18 (normal score 0; maximal deficit score 18). The higher the score, the worse was the sensorimotor function. These tests were performed by two observers blinded to the experiment on all rats before injury, and at days 1, 3, 5 and 7 after TBI.

**Evaluation of cerebral edema**

Fresh brains from the three groups were removed at 1 or 7 days post-injury. A 30-mg section of penumbral region from each extracted brain was obtained for the

![Fig. 1. Schematic representation of the area of direct injury induced by FPI. Detected areas A and B were obtained for the evaluation of brain water content and cytokine concentrations, respectively.](image-url)
evaluation of cerebral edema using a protocol described by Ward et al. (2011) (Fig. 1). To avoid artificial increases in cerebral water content, subdural hematoma was removed in all investigated rats before weighing the sections. These sections were immediately weighed and then dried at 60 °C for 72 h, and the dry weight was determined. The percentage of brain water content was measured as follows:

$$\text{Water content (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100\%.$$

**Multiplex assessment of 23 cytokines and chemokines**

Approximately 130-mg tissue was directly obtained from the injured cortex according to the procedure described previously (Ward et al., 2011) (Fig. 1), minced with a pellet pestle, and diluted in phosphate-buffered solution (PBS) (pH 7.4) at 1:5 (w/v) ratio. Homogenates were centrifuged at 12,000g for 15 min at 4 °C. Supernatants were removed and stored at −80 °C. Total protein concentration of the supernatant was quantified by bicinchoninic acid (BCA) assay (A054-3, Nanjing Jiancheng Bioengineering Institute, Nanjing, Olympus, Tokyo, Japan).

The levels of eotaxin, Granulocyte colony-stimulating factor (G-CSF); Granulocyte-macrophage colony-stimulating factor (GM-CSF); GRO/KC, interferon-gamma (IFN-γ), IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17, IL-18, IL-25, IL-28A, IL-28B, IL-29, IL-31, IL-32, IL-33, IL-35, IL-36γ, IL-37, IL-38, IL-40, IL-27, IL-28A, IL-28B, IL-29, IL-31, IL-32, IL-33, IL-35, IL-36γ, IL-37, IL-38, IL-40, IL-27, IL-28A, IL-28B, IL-29, IL-31, IL-32, IL-33, IL-35, IL-36γ, IL-37, IL-38, IL-40, IL-27, IL-28A, IL-28B, IL-29, IL-31, IL-32, IL-33, IL-35, IL-36γ, IL-37, IL-38

**GFAP immunofluorescence**

Rat brains were perfused transcardially with PBS and 4% cold paraformaldehyde in 0.1 M PBS (pH 7.4) followed by post-fixing in 4% paraformaldehyde for 2 days at room temperature. Two standard paraffin-embedded coronal blocks (3 mm) were obtained and sections (6 μm) including the center of the cerebral lesion were mounted on coated glass slides. De-paraffinized brain sections were incubated in 0.3% Triton X-100 for 10 min and then boiled in 1% citric acid buffer (pH 6.0) for 11 min in a microwave oven for antigen retrieval. The non-specific signals were blocked with 1% serum for 30 min. They were incubated overnight at 4 °C in the darkroom with a rabbit anti-glial fibrillary acidic protein polyclonal antibody (GFAP(H-50):sc-9065, Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted 1:50. After rewarmed to room temperature, the sections were incubated for 4 h with secondary ZymaxTM goat anti-rabbit immunoglobulin G (IgG) (H + L) fluorescent isothiocyanate-conjugated antibodies (FITC, dilution of 1:80; Invitrogen, Grand Island, NY, USA). The brain sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, dilution of 1:5000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 10 min. Each of the above steps was followed by three 5-min rinses in PBS. The staining was observed under Olympus BX63 light microscope, images were photographed using CellSens Standard software version 1.6 (Olympus, Tokyo, Japan). The number of GFAP-positive cells was counted three times in five different random regions in the ipsilateral cortex and hippocampus (400 ×) by two experienced pathologists in our laboratory. Finally, the number of astrocytes was averaged for each group.

**Statistical analysis**

Data were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). All values are expressed as mean ± SEM. Comparisons between the three different groups were statistically evaluated by a one-way analysis of variance (ANOVA) with Least Significant Difference (LSD) post hoc test. When normality tests failed, a Kruskal Wallis test was performed. In case of significance, intergroup differences were further analyzed using the Mann Whitney tests. Significance was based on two-tailed tests. A p value < 0.05 was considered statistically significant.

**RESULTS**

**Neurological score**

Fig. 2 shows the changes of sensorimotor function in rats after different treatments. The TBI group showed significant functional deficits at 1, 3, 5 and 7 days after TBI, compared with the sham group. In addition, the Tau group showed a significantly lower score than the
TBI group at 3, 5 and 7 days after TBI (p < 0.05), however, still at levels that were significantly higher than the sham group (p < 0.05).

Cerebral water content

Changes in the cerebral water content of the rats are shown in Fig. 3. TBI caused a significant increase in tissue water percentage 1 and 7 days after injury in the penumbra (p < 0.05). Taurine decreased cerebral water content 1 day after TBI, although not statistically significant (p > 0.05). The values of the Tau group were significantly lower compared with the TBI group 7 days after TBI (p < 0.05).

GFAP analysis

As is shown in Figs. 4 and 5, TBI significantly increased the number of GFAP-labeled cells in the ipsilateral cortex and hippocampus compared with sham group (p < 0.05). Taurine showed little effect on the number of GFAP-labeled cells on day 1 after TBI compared with the TBI group (p > 0.05). By day 7 post-injury, the number of GFAP-labeled cells in the Tau group was significantly decreased (p < 0.05).

Multiplex assessment of cytokines/chemokines

The effects of TBI and taurine on the intracerebral production of 23 cytokines/chemokines in the area of direct injury on days 1 and 7 post-injury are shown in Fig. 6. TBI induced increased concentrations of GRO/KC, IL-1α, IL-1β, IL-18, leptin, MCP-1, MIP-1α and RANTES on day 1 after injury, and eotaxin, G-CSF, GM-CSF, GRO/KC, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17, leptin, MCP-1, TNF-α and VEGF 7 days after injury, in the area of cortical injury (p < 0.05). Compared with the TBI group, a significant decrease in the production of GRO/KC and IL-1β occurred on day 1 after injury, and of eotaxin, G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-4, IL-6, IL-10, IL-12p70, IL-13, IL-17, lepton, MCP-1, TNF-α and VEGF 7 days after injury in the Tau group (p < 0.05), while elevated levels of RANTES on day 1 and increased levels of MIP-1α on day 7 were also noted (p < 0.05).

Within the sham group, concentrations of IL-1α, IL-4, IL-13, IL-18, and TNF-α were increased, and GRO/KC, IFN-γ, IL-1β, IL-2, IL-5, IL-6, IL-17, MCP-1 and MIP-1α were decreased 7 days after injury compared with the values on day 1. Furthermore, within the TBI group, concentrations of eotaxin, G-CSF, IFN-γ, IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-12p70, IL-13, IL-17, IL-18, TNF-α and VEGF were increased, and concentrations of GRO/KC, MCP-1, MIP-1α and RANTES were decreased 7 days after injury compared with the corresponding cytokines/chemokines on day 1. The cortical homogenate concentrations of IP-10 remained below detectable limits at all time-points in the three groups, it is not reported here.

DISCUSSION

In comparison with the sham group (control), animals with TBI exhibited a higher count of certain cytokines/chemokines and water content, increased accumulation of reactive astrogliosis in the ipsilateral brain, with evidence of neurological functional deficits. In clinical experiences there are at least three types of TBI, which included mild, moderate and severe types. Cortical injury, brain edema, reactive astrogliosis as well as neurological deficits were found in the present study, which can prove our model is a moderate TBI. Our previous literature has shown that taurine significantly increased the cerebral blood flow (CBF) of the cortex at 30 min and 24 h after TBI using the laser doppler flowmetry, and also...
significantly decreased the intercellular level of lactate, the ratio of lactate/pyruvate during 7 days after TBI using the microdialysis technology (Chang et al., 2012; Mo et al., 2012). Our present data demonstrated that taurine decreased the expression of certain cytokines on days 1 and 7 post-injury, reduced astrocyte activation and cerebral edema after 7 days, improved functional outcome at 3, 5 and 7 days after TBI. Treatment with drugs such as LF 18-1505T and baicalein that inhibit inflammation reduces brain edema and improves functional outcomes following TBI (Ivashkova et al., 2006; Chen et al., 2008). We therefore, speculated that taurine’s ability to attenuate the formation of cerebral edema in 7 days and improve functional outcomes following TBI may be associated with its anti-inflammatory effects. The anti-inflammatory actions of taurine seem to depend on its conversion to taurine chloramine (Tau-CL). Tau-CL inhibited the production of cytokines such as IL-1β, IL-6, and TNF-α (Marcinkiewicz et al., 1998; Park et al., 1998; Liu and Quinn, 2002; Georgia and Eunkyue, 2004). Since the cytokines are mostly secreted by reactive astrocytes, inhibiting reactive astrocytes by day 7 may result in the downregulation of cytokines in the present study. Consistent with previous reports, we found an almost similar cytokine pattern after TBI. The expression of specific inflammatory cytokines may vary depending on the animal models and the experimental conditions. However, within the sham groups, the levels of 14 cytokines/chemokines changed over time. One possible explanation is that saline injection and craniotomy may affect the production of cytokines/chemokines following TBI. It has been demonstrated that the production of GRO/KC and IFN-γ showed a significant increase in the craniotomy model compared with naive rats (Cole et al., 2011).

The proinflammatory cytokines, IL-1 and its related family member IL-18 are constitutively expressed in the brain at low concentrations. In the present study,
elevated levels of IL-1\(\alpha\) and IL-1\(\beta\) were observed in the TBI group on days 1 and 7 post-injury, and the level of IL-18 was increased only on day 1 post-injury. The elevated levels of brain IL-1\(\alpha\) were previously noted following FPI, indicating a rapid inflammatory response (Redell et al., 2013). In brain tissue perfusates obtained by in vivo microdialysis, IL-1\(\beta\) was increased within 60 min and peaked at 2 days after neurotrauma (Fassbender et al., 2000). Significantly higher IL-18 level was detected in the brain 7 days after close head injury (CHI), however, in human CSF elevated levels of IL-18 were observed up to 10 days after trauma (Yatsiv et al., 2002). Our data therefore, confirm the results from previous studies showing rapid expression and activation of the IL-1 family after TBI. The present study indicated that taurine significantly decreased the production of IL-1\(\alpha\) in 7 days after TBI, consistent with previous study that treatment with taurine attenuated the bleomycin-induced increases in IL-1\(\alpha\) in the bronchoalveolar lavage fluid (Gurujevalakshmi et al., 2000). We found that the level of IL-1\(\beta\) was reduced in the Tau group on days 1 and 7. IL-1\(\beta\) is the only cytokine decreased by taurine on both days following TBI. Researchers have demonstrated that neutralization of IL-1\(\beta\) modifies the inflammatory response and improves histological and cognitive outcome in mice following TBI (Clausen et al., 2009). This finding suggests that the neuroprotection of taurine may be related to ongoing suppression of IL-1\(\beta\) in our experiment.

Of the cytokines analyzed in this study, IL-4 is the primary regulatory cytokine that is responsible for the control of brain inflammation. Increased brain injury and worsened neurological outcome were observed in IL-4 knockout mice after transient focal cerebral ischemia, which was reversed by exogenous IL-4 (Xiong et al., 2011). IL-4 signaling may have reduced the inflammation,
suggesting that IL-4 may regulate brain inflammation by inducing the death of activated microglia in vivo and increasing neuronal survival (Park et al., 2005). In the present experiment, taurine attenuated the TBI-induced increase in IL-4 levels 7 days following surgery. The interaction of taurine with IL-4 signal pathway should be investigated in a TBI model to unravel the underlying mechanisms.

As an effective biomarker in severe brain injury, IL-6 is an important mediator of immune response and inflammation in CNS. Early studies demonstrated that levels of IL-6 in CSF were significantly higher than the plasma levels in patients who had suffered TBI (Kossmann et al., 1995). Taurine significantly decreased IL-6 levels in mice with SCI in a dose-dependent manner (Nakajima et al., 2010). Similarly, the present study indicated that taurine suppressed the TBI-induced increase in IL-6 levels 7 days following TBI. Since astrocytes were the predominant source of IL-6, the results support our histological findings showing that taurine attenuated astrocyte activation in 7 days.

Among cytokines, IL-10 also is an anti-inflammatory cytokine with an important role in suppressing inflammation and preventing exaggerated immune response. Systemic (intravenous or subcutaneous) but not intracerebroventricular administration of IL-10 improves neurological outcomes in rats with TBI (Knoblach and Faden, 1998). The result suggests that IL-10 modulated functional impairment associated with pro-inflammatory cytokines. The IL-10 level was quickly increased within the first 4 h and gradually increased over the last 20 h in the brain of rats with TBI (Kamm et al., 2006). In the present study, however, concentrations of IL-10 were below detectable limits in the three groups on day 1 after TBI. Similarly, IL-10 in the brain was not detected 24 h after mFPI (Redell et al., 2013). We found that IL-10 was elevated by day 7 following TBI. Taurine strongly decreased the IL-10 levels. These data suggest that a multiplex assay of inflammatory cytokines may accurately reflect the outcome of TBI.

IL-12p70, a heterodimeric cytokine composed of two disulfide-linked chains, p35 and p40, is produced primarily by antigen-presenting cells. Following cerebral injury, levels of IL-12p70 increased in the brain (Takamiya et al., 2007). However, levels of IL-12 were significantly lower than sham rats 3 and 24 h following FPI (Redell et al., 2013). IL-13, a well-known anti-inflammatory cytokine, suppressed the expression of...
pro-inflammatory mediators from activated microglia and macrophages. It was significantly decreased within 24 h of FPI (Redell et al., 2013). By contrast, Dalgard et al. found elevated IL-13 until three days post-injury in a controlled cortical impact model (Dalgard et al., 2012). The levels of IL-12p70 and IL-13 were increased in the TBI group by day 7 in the present study. IL-12p70 and IL-13, with opposing effects, were decreased by taurine, however, the mechanism requires additional investigation.

Interestingly, these results suggest that the expression of a unique cytokine in the brain after TBI was possibly associated with an imbalance between pro- and anti-inflammatory cytokines. Although inhibiting the production of anti-inflammatory cytokines such as IL-4, IL-10 and IL-13 following TBI, taurine mitigates the severity of brain damage.

Leptin is a 16-kDa protein that regulates energy balance through its effects in the brain on appetite and energy expenditure. In the present study, cortical leptin levels were increased on days 1 and 7 following TBI. Changes in serum leptin after TBI have also been reported (Wei et al., 2008). In addition, leptin level in the plasma was reported as a biomarker for predicting 6-month clinical outcome in children with TBI (Lin et al., 2012). All these findings imply that leptin may contribute to the inflammatory process of brain injury. Reduced leptin levels were noted on day 7 in the Tau group in the present study. It has been demonstrated that IL-1 increased leptin levels in patients with cancer (Janik et al., 1997). Similarly, TNF-α increased plasma leptin in cancer patients (Friedman and Halaas, 1998). These inflammatory pathways may mediate the decreased production of leptin observed for the first time in the Tau group, in vivo. Additional investigations are warranted to unravel the detailed mechanism.

Chemokines are small proteins that play a central role in TBI pathophysiology. They occur in two subfamilies: alpha-chemokines and beta-chemokines. Cerebral cortical lesions are associated with elevated mRNA levels of GRO/KC, MCP-1, MIP-1α, RANTES (Hausmann et al., 1998). In the same study, IP-10 peaked at 6 h and showed the most rapid decline, becoming undetectable a day after TBI. The protein levels of GRO/KC, MCP-1, MIP-1α and RANTES were increased after brain injury (Takamiya et al., 2007; Dalgard et al., 2012). In the present study, production of GRO/KC, MCP-1, MIP-1α, and RANTES was increased on the day following TBI. Chemokines were detected as early as few hours post-injury, indicating that the expression of these molecules was an early response following TBI. We may have missed the early increase in the present study. These chemokines are locally expressed by neuronal and glial cells. In addition to GRO/KC and MCP-1, eotaxin was also increased in 7 days. We found that taurine significantly raised RANTES level on day 1 and MIP-1α by day 7. Further, it also decreased GRO/KC on day 1, and eotaxin and MCP-1 in 7 days post-injury.

MCP-1 is the only chemokine, whose interaction with taurine has been investigated in vitro. It was demonstrated that MCP-1, secreted by activated alveolar macrophages, was inhibited by Tau-CL, mediated by the nuclear factor-κB (NF-κB) signaling pathway (Liu and Quinn, 2002). MCP-1, a monocyte chemoattractant produced by activated astrocytes after brain injury, plays an important role in the promotion of macrophage infiltration. The potential role of MCP-1 was investigated in MCP-1 knockout mice, suggesting that MCP-1 plays an important role in secondary brain damage (Semple et al., 2010). The present results showed that taurine affected chemokine production in the acute phase following TBI, extending our knowledge of taurine’s anti-inflammatory properties. However, additional investigation is required to fully understand the process.

As a well-known hematopoietic growth factor, G-CSF has been used in clinical practice for many years. Several reports showed the beneficial effect of G-CSF on functional recovery in rodents after TBI (Sheibani et al., 2004; Yang et al., 2010). However, no improvement in brain edema was noted in G-CSF-treated rats following TBI (Whalen et al., 2000). Taurine attenuated the TBI-induced increase in G-CSF in 7 days after TBI in the present study, although, the underlying mechanism is unclear. A previous study reported that the pro-inflammatory GM-CSF levels showed a significant increase from 17 min up to 122 h in the human cortex after TBI (Frugier et al., 2010). Surprisingly, concentrations of GM-CSF were below detectable limits in the three groups 1 day after TBI. Consistent with our finding, a recent study by Redell et al. showed that GM-CSF was also below the detection limit of the assay at 3 and 24 h post-injury using the same Luminex xMAP technology (Redell et al., 2013). The present study also found that GM-CSF was significantly raised in 7 days following TBI, and taurine strongly inhibited its expression. TNF-α, a pro-inflammatory cytokine, has been implicated in the pathogenesis of brain trauma. Significantly increased levels of TNF-α have been observed in CSF and plasma of patients with TBI (Ross et al., 1994). Vitarbo et al. has observed acute increases (1 h) in TNF-α protein and mRNA expression in injured cortex and ipsilateral hippocampus after experimental brain injury in rat (Vitarbo et al., 2004). In contrast to the previous study, we found a significantly higher TNF-α only 7 days after TBI. One possible explanation is that we have missed the acute increase after TBI. Taurine showed anti-inflammatory effect by reducing levels of TNF-α in various models of tissue injury (Zhang et al., 2010; Sun et al., 2012). Consistently, taurine inhibited TNF-α activity in 7 days in the current study, resulting in reduced cerebral edema and neurological improvement.

IFN-γ plays an important role in modulating inflammatory responses within the CNS. Protein and mRNA levels of IFN-γ increased quickly in human brain tissue following brain injury (Holmin and Hojeberg, 2004; Frugier et al., 2010). In the present study, however, IFN-γ levels were elevated in 7 days after TBI. The inflammatory response correlated with the intensity of the trauma and exposure to stimuli such as saline injection in the current study. IFN-γ levels were also robustly inhibited by taurine in 7 days. Astrocytes
produce IFN-γ induced by TNF-α, so the reduced IFN-γ may be related to the decreased TNF-α in 7 days (Xiao and Link, 1998).

VEGF is a vascular growth factor, which induces neoangiogenesis and vascular permeability. It also plays an important role in the pathogenesis of chemotaxant and inflammation. VEGF upregulation has been implicated in the development of cerebral edema. RNA and protein expression of VEGF were detected in or adjacent to the injury site from day 1 after contusion, with a peak expression after 4–6 days (Skold et al., 2005). Our present study also showed that VEGF levels were significantly increased by day 7 following TBI. Taurine decreased VEGF levels in 7 days after injury, and thereby reduced cerebral edema in the Tau group.

CONCLUSION

In summary, we conclude that taurine reduced the expression of inflammatory cytokines, reactive astrogliosis, cerebral edema and improved neurological function following TBI. Further investigations are needed in multiple brain regions and at multiple time-points after injury to determine the underlying mechanisms of action and interaction with cytokines.

Acknowledgments—This work was supported by the National Natural Science Foundation of China (30973089), Tianjin Research Program of Application Foundation and Advanced Technology (10JCYBJC12200) and fund project of Tianjin Municipal Health Bureau of Science and Technology (2012KR09).

REFERENCES


