Alterations of blood IL-8, TGF-β1 and nitric oxide levels in relation to blood cells in patients with acute brain injury

Elham Hadidi, Mojtaba Mojtahedzadeh, Mohammad Hassan Paknejad, Shekoufeh Nikfar, Mohammad Jafar Zamani, Mohammad Ali Sahraian, Behzad Eftekhar, Mohammad R Khajavi, Atabak Najafi, Mahtab Ghaffarzadeh, Righieh Eftekhari, Venus Soleimani, Hadi Esmaily, Mohammad Reza Rouini & Mohammad Abdollahi†

†Author for correspondence Tehran University of Medical Sciences (TUMS), Laboratory of Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Pharmaceutical Sciences Research Center, Tehran 14155 6451, Iran Tel.: +98 216 695 9104 Fax: +98 216 695 9104 mohammad@sina.tums.ac.ir

Background: Acute brain injury (ABI) disrupts homeostasis in tissue brain. Inflammation has an important contributory role in the pathogenesis of the disease and blood cell count alteration has been shown. The clinical course is variable, and the factors or markers available for predicting survival or the functional situation of these patients are limited. **Aim:** The aim of this study was to measure the serum levels of interleukin (IL)-8, transforming growth factor (TGF)-β1 and nitric oxide (NO) in patients with ABI (at days 1, 2, 3 and 7) and to study their relationships to blood cell counts. **Methods & results:** A total of 25 patients were included in the study and 15 healthy subjects were chosen as a control group. Expression of IL-8 and TGF-β1 and production of NO were higher in ABI patients at each of the time points as compared with the control group. In the case of TGF-β1, the increase at time points 3 and 7 was greater than time points 1 and 2. White blood cells were increased significantly (12130 ± 1372 cells/μl; $p < 0.01$). A huge decrease in platelet counts was also observed (202 ± 14 cells/μl; $p < 0.01$). Interestingly, no significant correlation was found between the serum levels of these mediators and blood cell counts (mainly considered leukocytes and platelets). **Discussion & conclusion:** This study shows dramatic increase in the serum levels of three important mediators involved in inflammation and progression of ABI. We showed that there is no correlation between the serum levels of these mediators, leukocytes and platelets and probably they act independently from each other.

Keywords: acute brain injury, biomarker, cytokine, interleukin, nitric oxide, transforming growth factor

Acute brain injury (ABI) is one of the major causes of morbidity and mortality among the population, especially in younger patients [1]. Inflammation is one of the four mechanisms defined and associated to ABI [1]. In this regard, several compounds and mediators with different actions related to inflammation have been known. In fact, proinflammatory cytokines play crucial role in brain trauma [1,2].

Interleukin (IL)-8, a proinflammatory and chemotactic cytokine for neutrophils [3–5], can be synthesized by a wide range of cell types including monocytes, macrophages, endothelial cells, neutrophils and astrocytes [6–9] and is produced in a variety of diseases of the CNS. High concentrations of IL-8 have been reported in the cerebrospinal fluid of infants and children after brain trauma [1]. Thus, IL-8 may represent a pivotal cytokine in the pathology of brain injury.

Transforming growth factor (TGF)-β1, a cytokine produced by activated T cells, mononuclear phagocytes and other cells whose principal actions are to inhibit the proliferation and differentiation of T cells, inhibits the activation of macrophages and counteracts the effects of proinflammatory cytokines, especially if present before cell activation.

The role of nitric oxide (NO) in apoptosis (programmed cell death) and necrosis (excitotoxic cell death) may have implications in post-traumatic events. Interestingly, equivocal actions of NO in traumatic events (such as ABI) have been reported. Our recent investigations showed interesting results on the roles of cytokines such as NO and growth factors in the pathology or prognosis of many acute and chronic illnesses [10–20].

The objective of the present study was to assay the amounts of IL-8, TGF-β1 and NO in the serum of ABI patients and to elucidate whether there is an association between their concentrations to each other and also to several blood cells involved in inflammation.

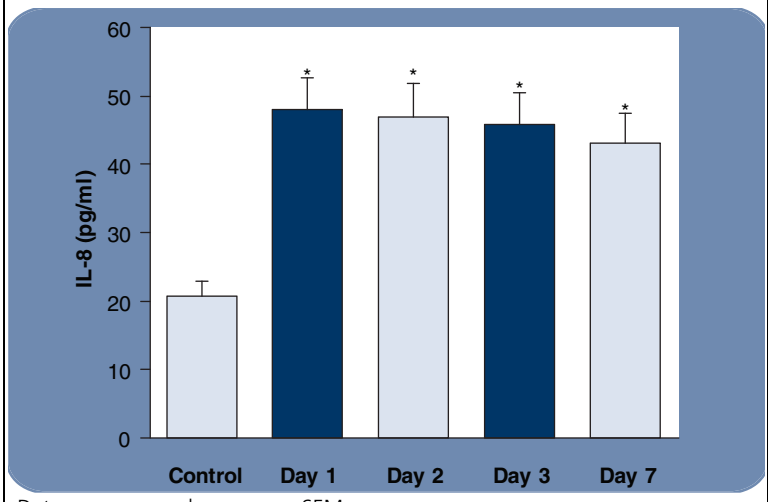
Materials & methods

Subjects & sample collection

A total of 25 patients, 14 male, 11 female, with an age of 51 ± 23 years (mean ± SD, range 18–85 years) with ABI, who were admitted within 24 h after onset to the Neurology and Neurosurgical Departments of Sina University Hospital (Tehran, Iran) between April 2004 and January 2005, were included in the study. Patients with: clinical evidence of acquired infection after admission, a history of

10.1586/14750708.3.3.399 © 2006 Future Drugs Ltd ISSN 1475-0708

Figure 1. Serum level of IL-8 in patients with acute brain injury.



Data are expressed as mean ± SEM.
*Difference between patients and control groups is statistically significant at $p < 0.05$.
IL: Interleukin.

malignancy, autoimmune disease or myocardial infarction, concurrent major cardiac, renal or hepatic diseases, transient ischemic attack or stroke due to aneurysmal rupture, arteriovenous malformations, moyamoya disease, and other vascular malformations, and recent (within 1 month) history of head trauma were excluded from the study.

The study was performed with the approval of TUMS ethics committee on human research. A total of 15 age- and sex-matched healthy subjects were recruited as a control group. **Table 1** shows a summary of some of the characteristics of the patients studied including age, sex, risk factors, Glasgow Coma Score (GCS), counts of blood cells, and the final outcome. Peripheral blood was obtained from each patient at four time points on days 1, 2, 3 and 7 after onset of ABI. Blood samples were taken within 24 h (as soon as the patients were examined), and at days 2, 3, and 7 after admission through (from) indwelling intravenous catheters. Samples were centrifuged within 30 min at 1500 g for 10 min, and the sera stored at -70°C until assayed. Peripheral leukocytes and platelets were simultaneously counted at the four time points mentioned. A number of routine blood parameters, such as cholesterol and hemoglobin, were also determined (only at the time of admission). Patients were questioned for important risk factors such as diabetes mellitus, myocardial infarction and hypertension. Two patients had a previous history of opium addiction.

Measurement of serum IL-8, TGF-β1 & NO

Serum levels of IL-8, TGF-β1 and NO were measured in duplicate using commercially available quantitative enzyme-linked immunosorbent assay kits (Quantikine®, R&D System, Germany).

Statistical analysis

Data are expressed as the mean ± SEM. For comparison of IL-8, TGF-β1 and NO serum concentrations between patients and the matched healthy subject control group Student's t-test was used. For evaluation of correlations between quantitative variables, linear regression analysis was used and subsequently, Spearman's p and r were calculated. In each case, a $p < 0.05$ was considered statistically significant. Analysis was carried out using the SPSS statistical software package, version 10.

Results

Clinical & demographic data

A total of 25 ABI patients (15 men and 10 women) were evaluated. Fourteen were defined as having traumatic brain injury, 11 had spontaneous intracerebral (intraparanchymatous) hemorrhage. There were no differences in clinical scores or in the male:female ratio between the traumatic brain injury group and patients with hemorrhage. Patients were classified according to brain computed tomography results into those with hemorrhage in basal ganglia (27%), posterior fossa hemorrhage (18%), lobar hemorrhage (45%), and intraventricular hemorrhage (72%). In six patients (54%), hematoma volume was greater than or equivalent to 20 ml and five patients had a hematoma volume of less than 20 ml.

Patient characteristics, including initial severity of injury assigned By GCS score, hematological data taken at the time of first sampling and final outcome after 7 days are shown in **Table 1**.

There were no signs or symptoms of infection in any of the patients in the 7-day study period, and all daily serum creatinine levels were within the normal range during this period as well.

The mean levels of IL-8 in patients with ABI were 47.97 ± 4.31 pg/ml at day 1, 47 ± 4.23 pg/ml at day 2, 45.95 ± 4.13 pg/ml after 3 days, and 43.02 ± 3.87 pg/ml after 7 days. All of these values were statistically significant as compared with the control values (20.75 ± 1.86 pg/ml) (**Figure 1**). The level of IL-8 (the mean calculated for each patient) was not correlated with the number of leukocytes ($r = 0.287$, $p = 0.321$), number of platelets

Table 1. Characteristics of study populations.

Parameter	TBI group n = 14	Hemorrhage group n = 11	Total n = 25
Sex M/F	13/1	2/9	15/10
Age range (mean years)	18–70 (35.9)	46–85 (71.7)	18–85 (51.7)
Hypertension	0	9	9
Diabetes mellitus	0	2	2
Current smoking	5	1	6
Initial GCS (mean ± SD)	12.2 ± 3.5	10.6 ± 4.3	11.52 ± 3.8
Leukocytes/mm^3 (mean ± SD)	16200 ± 6645	10722 ± 354	13959 ± 613
Monocytes/mm3 (mean ± SD)	739 ± 360	648 ± 266	699 ± 319
PMN/mm^3 (mean ± SD)	13921 ± 6937	8787 ± 3453	11689 ± 6156
Lymphocytes/mm^3 (mean ± SD)	1539 ± 1373	1702 ± 631	1610 ± 1095
Platelets (x103)/mm3 (mean ± SEM)	244 ± 85	200 ± 35	225 ± 70
Mortality at day 7	14	54	32

GCS: Glascow Coma Scale score; ICH: Intracerebral hemorrhage; PMN: Polymorphonuclears; TBI: Traumatic brain injury.

(r = 0.413, p = 0.143), TGF-β1 level (r = 0.172, p = 0.556), or the serum level of NO (r = 0.340, p = 0.234).

The mean values of serum TGF-β1 were significantly higher compared with those of the control patients (93.5 ± 8.41 pg/ml) at all time points ($p < 0.05$). The mean levels of TGF-β1 in patients with ABI were 473 ± 42.5 pg/ml at day 1, 458 ± 41.2 pg/ml at day 2, 678 ± 61 pg/ml after day 3 and 676 ± 60.8 pg/ml after day 7 (**Figure 2**). There was no correlation between the serum concentration of TGF-β1 with the number of leukocytes (r = 0.064, p = 0.829), number of platelets (r = 0.153, p = 0.602), or the serum level of NO (r = 0.367, p = 0.197).

The mean levels of NO were 36 ± 3.24 pg/ml at day 1, 40 ± 3.6 pg/ml at day 2, 41 ± 3.69 µmol/L after day 3 and 40 ± 3.69 pg/ml after day 7. All of these values were statistically significant as compared with the control values (29 ± 2.61 pg/ml) (**Figure 3**). The serum levels of NO were not correlated with the number of leukocytes (r = 0.076, p = 0.796) and number of platelets (r = 0.176, p = 0.548)

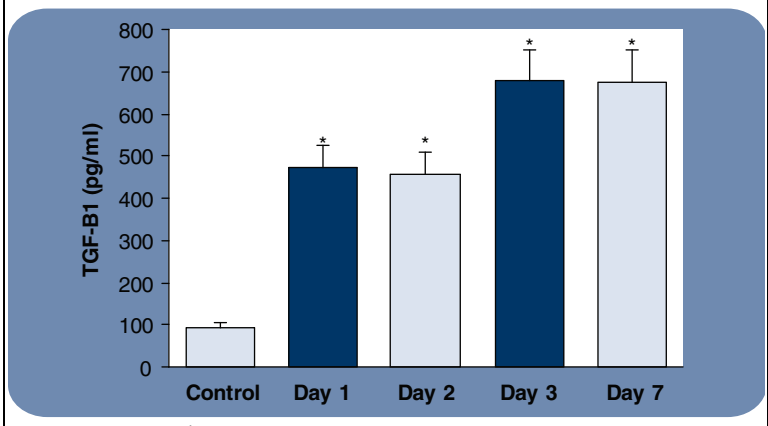
Discussion

Inflammatory response, characterized and modulated by the release of several cytokines with proinflammatory and anti-inflammatory functions [2], has a major contribution to secondary damage after ABI [1]. After CNS injury the blood–brain barrier (BBB) becomes leaky, which facilitates the entry of activated circulating immune cells into the CNS [21–23]. Systemic cells, as well as those resident in the CNS, contribute to intrathecal production of cytokines. Overall, there appear to be both acute detrimental and subacute/chronic beneficial aspects of inflammation in ABI [1].

We found that the levels of IL-8, TGF-β1 and NO were increased in the serum of ABI patients. However, the time at which these mediators reached their peak and patterns of rise and fall in serum concentrations were different; both NO and TGF-β1 peaked within 3 days but they showed rather different patterns when the concentrations were measured serially. IL-8 showed a peak at the acute phase; thereafter the level decreased. Leukocytosis and a decrease in platelet counts were also seen during the study.

TGF-β1, a disulphide-linked nonglycosylated homodimer [24] immunomodulatory cytokine [25,26], is synthesized by many cell types, including astrocytes, neurons, microglia [27–29], platelets [30,31] and peripheral leukocytes [25,30,31]. It can inhibit the actions of inflammatory cytokines such as TNF-α [32] and modulates a variety of functions including replication, differentiation, migration, adhesion and apoptosis in different cell types during developmental, oncogenic, immunologic and angiogenic processes [33,34]. TGF-β can also inhibit the migration of leukocytes through the BBB [35]. A neuroprotective role for TGF-β has been shown [36,37]. Biphasic (stimulatory and inhibitory) role of TGF-β following traumatic injury in the CNS is possibly due to the differential expression of TGF-βRI and TGF-βRII on brain endothelial cells [38]. Despite the reports on contradictory changes in TGF-β1 concentrations in serum of patients with

Figure 2. Serum level of TGF-β 1 in patients with ABI.

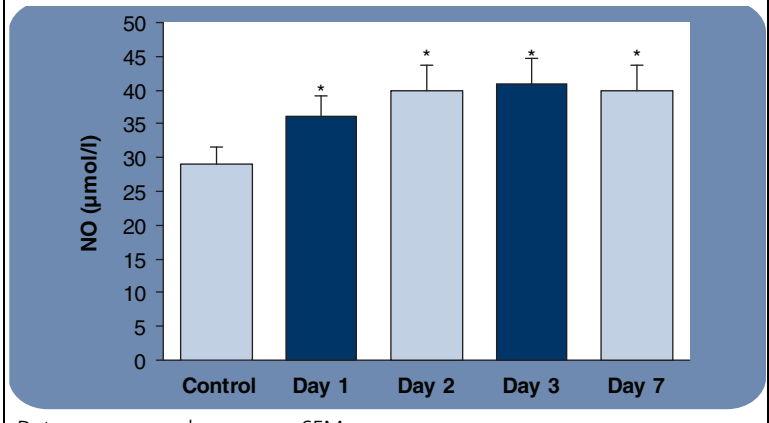


Data are expressed as mean ± SEM.
*Difference between patients and control groups is statistically significant at $p < 0.05$.
ABI: Acute brain injury; TGF: Transforming growth factor.

brain injuries [39–41], we showed that levels of TGF-β1 increases over time in patients with ABI. Previous studies have shown a reduction of platelets after brain injuries [42] and we confirmed this reduction in ABI patients. Leukocytosis observed in the study could be attributed to the inhibitory effects of TGF-β1 on migration of these cells through BBB that could result in accumulation of leukocytes in peripheral compartment.

IL-8, a potent chemotactic [3–5] and proinflammatory cytokine [3], can be synthesized by monocytes, macrophages, endothelial cells, neutrophils and astrocytes [6–9]. IL-8 has been associated with severe BBB dysfunction after brain injury [43]. It displays a neurotrophic activity in the CNS and may also regulate other activities here [44,45]. Induction of neurotrophins, such as the nerve growth factor, can be triggered by IL-8 after ABI [46,47]. Presumably, these cytokines could play a critical role in the neuronal plasticity which is damaged after ABI and plays an important role in mediating beneficial long-term effects on recovery [1]. Release and synthesis of IL-8 in the CSF and serum of patients after brain events such as ABI has been shown [46,48–50]. Concentrations in CSF are higher than in the serum [51,52]. This indicates that IL-8 is a key mediator of neuroinflammation [52]. In addition, it seems likely that high concentrations of IL-8 be associated to a worse prognosis after ABI. In this study we showed serially decrease of the levels of IL-8 in serum of ABI patients after a huge increase in the acute phase. We hypothesize that an early synthesis and release of IL-8 in CNS and then disruption of BBB could occur during the acute phase of ABI. Thereafter, regeneration of the barrier occurs and IL-8 concentrations falls.

Figure 3. Serum level of NO in patients with ABI.



Data are expressed as mean ± SEM.
*Difference between patients and control groups is statistically significant at $p < 0.05$.
ABI: Acute brain injury; NO: Nitric oxide.

NO is a multifunctional chemical messenger in the brain and it can have both beneficial and detrimental effects in disease states, including ABI [53]. Physiologically, the content of NO is highest in the brain where it can have important and diverse roles in pathological processes [53]. Activation of constitutive NO synthase, which leads to NO production, peroxynitrite formation and resultant DNA damage, is one of the mechanisms involved in the evolution of secondary damage after ABI (through cytotoxic effects) [1]. Mice deficient in inducible NO synthase (iNOS) exhibit impaired long-term outcome while a detrimental role for iNOS early after trauma has been shown [54]. There are two periods of time after injury when NO accumulates in the brain, immediately after injury and then again several hours–days later [53,54]. In our study, we showed that NO levels reached a peak within 3 days after ABI. The pattern observed in serum is different from the pattern in the CNS. One of the limitations of the study was the short duration of follow-up of the patients (7 days). In addition, the control group was chosen from healthy subjects. Thus, it is hard to state that ABI is an inflammatory disease. Maybe the cytokine levels increase due to any acute illness per se. Ideally, a control group with patients sufferings from some acute illness that does not influence the CNS would be preferable. In such mild injuries, the leakiness of the BBB is questionable and may not permit passage of these

cytokines. Therefore, any influence on the CNS in the absence of, for example, CSF levels, is highly speculative, but obtaining CSF from these patients would not be ethical.

Further studies with more included patients are required for master evaluation of changes in concentrations of the cytokines in the serum of patients with ABI.

Acknowledgement

This study was granted by Pharmaceutical Sciences Research Center of TUMS.

Highlights

- Inflammation is one of the four mechanisms defined and associated to acute brain injury. Proinflammatory cytokines play a crucial role in brain trauma.
- The levels of interleukin (IL)-8, transforming growth factor (TGF)-β1 and nitric oxide (NO) are all increased in the serum of acute brain injury (ABI) patients. However, the time at which these mediators reach their peaks and patterns of rise and fall in serum concentrations are different. Both NO and TGF-β1 were shown to peak within 3 days but demonstrated different patterns when the concentrations were measured serially. IL-8 showed a peak at the acute phase; thereafter the level decreased.
- Leukocytosis and a decrease in platelet counts are also seen in ABI patients and are not correlated with the elevation of cytokines.
- Leukocytosis observed in the study could be attributed to the inhibitory effects of TGF-β1 on migration of these cells through the blood–brain barrier that could result in accumulation of leukocytes in the peripheral compartment.
- IL-8 is a key mediator of neuroinflammation and high concentrations of IL-8 could be associated to a worse prognosis after ABI.
- NO is a multifunctional chemical messenger in the brain and can have both beneficial and detrimental effects in disease states, including ABI.
- Inflammatory response, characterized and modulated by the release of several cytokines with proinflammatory and anti-inflammatory functions, has a major contribution to secondary damage after ABI.

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Affiliations

Elham Hadidi, PharmD,
Resident of Clinical Pharmacy
Tehran University of Medical Sciences (TUMS),
Faculty of Pharmacy, Tehran 14155-6451, Iran
Tel.: +98 216 699 104
Fax: +98 216 695 9104
mohammad@tums.ac.ir

Mojtaba Mojtahedzadeh, PharmD, BCPS,
Associate Professor of Clinical Pharmacy
TUMS, Faculty of Pharmacy and,
Pharmaceutical Sciences Research Center,
Tehran 14155-6451, Iran

Mohammad Hassan Paknejad, MD,
Resident of Neurology, TUMS,
Sina University Hospital,
Faculty of Medicine,
Tehran 14155-6451, Iran

Shekoufeh Nikfar, PharmD, MPH,
Drug Evaluation Committee,
Food & Drug Organisation,
MOH and Research Assistant, TUMS,
Pharmaceutical Sciences Research Center,
Tehran 14155-6451, Iran

Mohammad Jafar Zamani, PharmD,
Research Assistant TUMS,
Pharmaceutical Sciences Research Center,
Tehran 14155-6451, Iran

Mohammad Ali Sahraian, MD,
Assistant Professor of Neurology, TUMS,
Sina University Hospital, Faculty of Medicine,
Tehran 14155-6451, Iran

Behzad Eftekhar, MD,
Assistant Professor of Neurology, TUMS,
Sina University Hospital, Faculty of Medicine,
Tehran 14155-6451, Iran

Mohammad R Khajavi, MD,
Assistant Professor of Anesthesiology &
Critical Care Medicine,
TUMS, Faculty of Medicine,
Tehran 14155-6451, Iran

Atabak Najafi, MD,
Assistant Professor of Anesthesiology &
Critical Care Medicine,
TUMS, Faculty of Medicine,
Tehran 14155-6451, Iran

Mahtab Ghaffarzadeh, CCRN
TUMS, Sina University Hospital,
Tehran 14155-6451, Iran

Righieh Eftekhari, CCRN
TUMS, Sina University Hospital,
Tehran 14155-6451, Iran

Venus Soleimani, CCRN
TUMS, Sina University Hospital,
Tehran 14155-6451, Iran

Hadi Esmaily,
Research Assistant, TUMS,
Pharmaceutical Sciences Research Center,
Tehran 14155-6451, Iran

Mohammad Reza Rouini, PharmD, PhD,
Associate Professor of Pharmaceutics,
TUMS, Faculty of Pharmacy and Pharmaceutical
Sciences Research Center, Tehran 14155-6451, Iran

Mohammad Abdollahi, PharmD, PhD,
Professor of Toxicology and Pharmacology,
TUMS, Laboratory of Toxicology,
Department of Toxicology & Pharmacology,
Faculty of Pharmacy and,
Pharmaceutical Sciences Research Center,
Tehran 14155-6451, Iran