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Cerebrospinal IL-10 Concentration is Elevated in Non-survivors as Compared to Survivors after Severe Traumatic Brain Injury

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Abstract
Objective: The intrathecal posttraumatic inflammation contributes to secondary brain damage as well as to the induction of neuroreparative mechanisms. In this context release of interleukin-10 (IL-10) has been reported to play a major role. However, initial IL-10 concentration in CSF remains incompletely characterized. Therefore, the aim was to analyze Il-10 in CSF and serum of patients early after TBI.
Methods: For control, 10 volunteers receiving spinal puncture were enrolled. In patients with severe TBI (GCS <8pts.), CSF and serum was drawn within 90 ± 45min after intraventricular catheter insertion (0h), as well as 12h, 24h and 48h after TBI. Albumin for assessing Blood-Brain-Barrier (BBB) function and IL-10 (IMMULITE, DPC Biermann, Bad Nauheim, Germany) were analyzed.
Results: 23 patients were enrolled. 15 survived and 8 deceased within 24h. In controls, CSF IL-10 was below detection limit (<5pg/ml). In contrast, IL-10 was elevated significantly in non-survivors at 0h vs. survivors and controls (30 ± 6 vs. 9 ± 1 vs. <5pg/mL). This was accompanied by a significant increase of serum IL-10 in both groups at 0h vs. controls (survivors: 30 ± 6pg/mL, non-survivors: 48 ± 8pg/mL, controls: 10 ± 7pg/mL, $p < 0.001$). Survivors revealed signs of a mild BBB dysfunction during the entire observation period. In contrast, non-survivors presented a severe BBB breakage.
Conclusions: We demonstrated an analysis of IL-10 CSF and serum concentration after TBI. These data support an intrathecal IL-10 synthesis. Although the significant increase of IL-10 might indicate a bad outcome of TBI, responsible mechanisms still have to be elucidated.

Introduction

The morbidity and mortality of patients suffering from traumatic brain injury (TBI) is influenced by the primary brain damage, expressed in mechanical trauma to the cerebral tissue as well as the development of secondary brain damage, expressed by edema, swelling and increase of intracranial pressure [1]. In this context, inflammatory response within the intrathecal compartment was proposed to contribute additionally to the secondary brain damage [2-6]. Interleukins seem to play a major role in the modulation of pathophysiological alterations after TBI. Especially, the anti-inflammatory cytokine interleukin-10 (IL-10) came into the focus of research, since several experimental studies gave significant hint towards its anti-inflammatory potential [6-12]. With regard to brain injury, local administration of IL-10 at the injured site attenuated the number and the hypertrophic state of reactive astrocytes and microglia and diminished TNF-α mRNA expression [11]. However, Bell et al. first reported that higher IL-10 levels in CSF of brain injured children were correlated to increased mortality.

Though, the initial posttraumatic IL-10 concentration in CSF obtained from adult patients, suffering from severe TBI remains incompletely characterized. Hence, the aim of this study was to analyze IL-10 secretion patterns in CSF and systemic circulation of TBI patients and to correlate the obtained results to the function of the Blood-Brain-Barrier as well as the clinical outcome.

Patients and Methods

Study Design and Patient Collective

Between 11/2003 and 11/2005 all patients presenting with isolated severe traumatic brain injury were enrolled. For negative control, CSF and serum samples were obtained from healthy patients, receiving spinal anesthesia for a standard orthopedic procedure. The study protocol was approved by the University's Medical Board of Ethics (reference no. 330/03). Enclosure criteria were an initial GCS ≤8 pts, age >18years, interval between trauma and sample collection ≤90 ± 45min and radiological signs of intracranial hemorrhage (ICH) on the initial CCT scan. Exclusion criteria were a history of pre-existing neurological, malign or inflammatory disease. Written informed consent for participating in the study was either obtained from the patient after neurological recovery or from a legal representative. Demographic, clinical information (i.e. gender, age, GCS) and history of systemic diseases was recorded using standardized data collection forms.

Clinical Management Protocol

After trauma resuscitation and initial CCT, intraventricular drainage catheters (IDC) were inserted into the frontal horn of lateral ventricle in all patients (within 90 ± 45min after admission) for continuous monitoring of ICP as well as for drainage of CSF. Patients were admitted to the intensive care unit (ICU) and treated in accordance with the guidelines of the Brain Trauma Foundation [13]. No corticosteroid was administered. All patients were treated with catecholamines (arterenol) and some received higher doses due to circulatory instability.

Sampling Procedures and Analysis of Interleukin-10 (IL-10)

After IDC-insertion, 3mL drained intraventricular CSF and paired 5mL of peripheral blood were collected. The first sampling time point was immediately after insertion of the intraventricular drainage catheter (within 90 ± 45min after TBI). Further CSF and serum samples were obtained at 12h, 24h and 48h standardized to time point of TBI. The CSF samples were centrifuged to remove cellular debris and the supernatant was immediately processed. IL-10 levels were subsequently measured using a completely automated chemiluminescence quantification system (IMMULITE, DPC Biermann, Bad Nauheim, Germany).

Assessment of Blood Brain Barrier (BBB) Function

The Reiber quotient (ratio of CSF and serum albumin, $Q_{albumin}$) was calculated to determine BBB-leakage, as described before [14]. The breakage of the BBB was assessed as follows: $Q_{albumin}$ values below $7x10^{-3}$ were considered as normal, values between 7x10-3 and $1x10^{-2}$ as sign of mild dysfunction, values between $1x10^{-2}$ and $2x10^{-2}$ as moderate dysfunction, and levels above $1x10^{-2}$ as severe dysfunction. Albumin levels were measured using standardized turbidimetric assay (Cobas Integra® Albumin; Roche® Diagnostics; Mannheim, Germany).

Data Analysis

The Sigma Stat® 3.0 software package (SPSS® Inc., Chicago, USA) was employed for all statistical analysis. Statistical significance between groups was determined Mann-Whitney U-test. In order to analyze the influence of functional parameters of the BBB on the IL-10 levels in CSF, linear regression analysis of IL-10 in CSF and $Q_{albumin}$ values of all patients was performed. A p-value of less than 0.001 was considered to be statistically significant. Data are given in mean ± SEM.

Results

Patient Collective and Clinical Data

CSF and serum were obtained from 10 control patients (5 male, 5 female; 44 ± 9years mean age), who received spinal anesthesia for a standard orthopedic procedure of the lower extremity.

In total, 23 patients with isolated severe traumatic brain injuries were enrolled (15 male, 8 female; aged 44 ± 2years, mean ± SD). Patients were sub-grouped into surviving (group I, n = 15) and deceased (group II, n = 8) patients. Patients in group II all deceased within 24h after TBI, due to untreatable brain swelling and/or brain stem herniation. The main reasons for TBI were traffic accidents or fall from a height. The groups did not differ with respect to gender, age or trauma mechanism. There was no statistical correlation of IL-10 levels and dosage of catecholamines.

Interleukin 10 (IL-10) Levels in CSF

The IL-10 concentration in lumbar CSF, obtained from negative control individuals was below the detection level in all patients (<5pg/mL).

In contrast, the IL-10 concentration in CSF of TBI patients was slightly elevated in the survivor group I on admission (9 ± 1pg/mL). IL-10 levels in group II were significantly elevated on admission (30 ± 6 pg/mL). The IL-10 concentrations in CSF of the survivor group decreased already 12h after TBI below detection level and remained there until the end of the observation period. In contrast, the concentration of the non-survivor group remained significantly increased 12h after TBI (8 ± 2pg/mL) and finally dropped below detection level 24h after TBI). In this respect it appears noteworthy, that the number of patients in the deceased group is not constant due to bad clinical outcome; the detailed number of samples was on admission: n = 8; 12h: n = 5; 24h: n = 3; 48h: n = 0. Figure 1 demonstrates the IL-10 concentration in CSF of both groups as compared to controls.

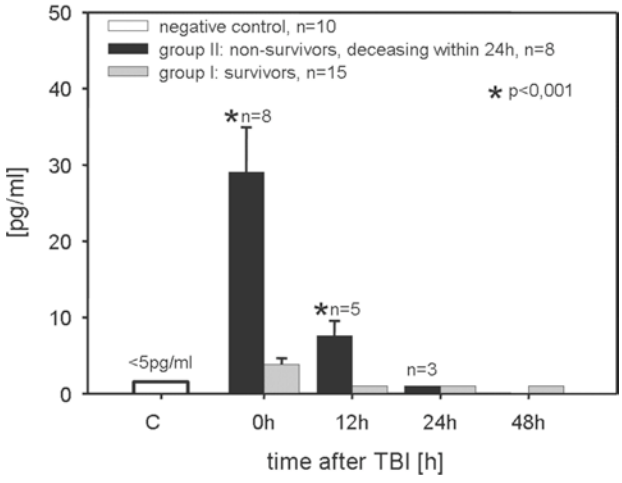

Fig. 1. CSF IL-10 levels.

Interleukin 10 (IL-10) Levels in Serum

In serum of the negative control group concentrations of IL-10 accounted for 10 ± 7pg/mL. In contrast, serum IL-10 levels were significantly elevated on admission in both groups (group I: 30 ± 6pg/mL, group

II: 48 ± 8pg/mL). IL-10 levels in the survivor group decreased continually to 27 ± 6pg/mL (48h) and were thereby significantly elevated during the entire observation period. The IL-10 levels of the non-survivor group increased furthermore to 78 ± 14pg/mL after 12h. The concentration of IL-10 at 24h after TBI accounted for 90 ± 15pg/mL in serum of patients in the non-survivor group. The dynamics of serum IL-10 of both groups and negative controls are depicted in Figure 2.

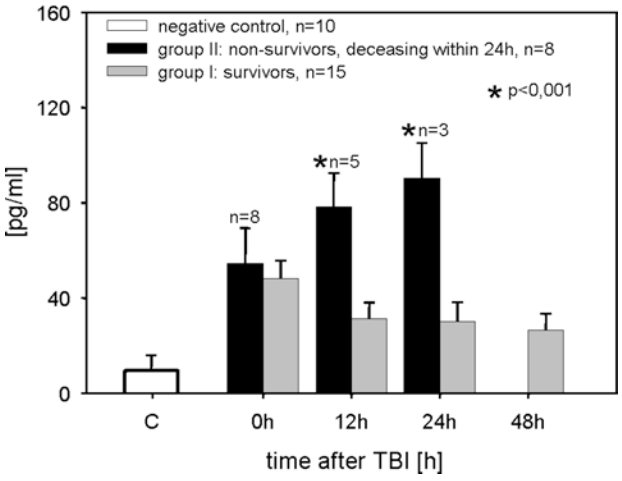

Fig. 2. Serum IL-10 levels.

Blood Brain Barrier (BBB) Function

The $Q_{albumin}$ ratio was 8.5 ± 9 x10^{-3} in group I (survivors) on admission and remained within ranges of a mild dysfunction during the entire observation period. In contrast, the $Q_{albumin}$ ratio of group II (non-survivors) accounted for 29 ± 15 x10^{-3} on admission, therefore revealing a severe breakage of the BBB and remained within ranges of severe disruption during the entire observation period (see Fig. 3). No significant correlation between CSF IL-10 and $Q_{albumin}$ values was detected using linear regression analysis.

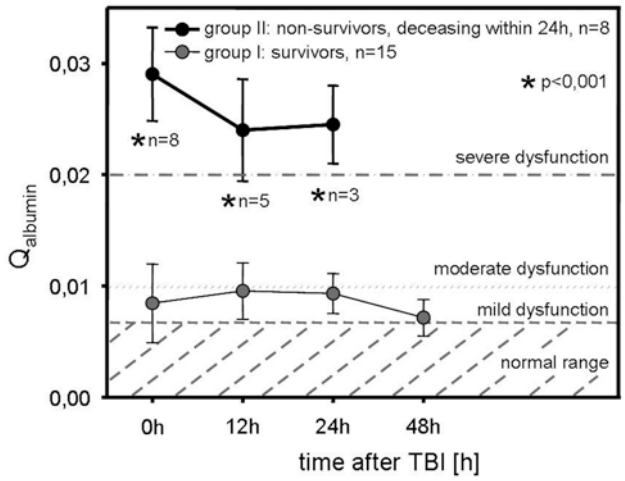

Fig. 3. Blood-Brain-Barrier functon.

Discussion

In the present study, we demonstrated for the first time CSF and serum IL-10 concentrations, obtained within 90min after trauma in TBI patients. We focused especially on the initial 48 hours after the traumatic insult and demonstrated a highly significant difference in comparing IL-10 CSF and serum levels of patients surviving TBI and patients who died within this acute period. This is substantially new information in comparison to other studies on IL-10, previously published [15-20].

Study Protocol

A direct approach to human cerebral posttraumatic inflammatory processes is particularly difficult due to ethical reasons. Therefore, we assessed the posttraumatic anti-inflammatory reaction in the CNS by determining IL-levels in the CSF. This is only possible in patients, having sustained the placement of a diagnostic and therapeutic intraventricular catheter, as reported before [15, 21, 22]. The treatment protocol of the patients was carefully analyzed since in particular catecholamines may be potential inducers of IL-10 as previously reported [23, 24]. All patients were treated with catecholamines (arterenol) and some received higher doses due to circulatory instability. However, we found no statistical correlation of IL-10 levels and dosage of catecholamines.

Concerning the time points of sample acquisition we drew samples immediately after admission to our trauma shock unit, i.e. 90 ± 45min after TBI. In the further course samples were drawn 12, 24, 48 and 72h standardized to the time point of TBI.

IL-10 in CSF and Serum

However, Bell et al. first reported that higher IL-10 levels in CSF of brain injured children were correlated to increased mortality [20].

Patients in the deceased group, presenting a significantly elevated CSF IL-10, all suffered from highly elevated ICP (>35mmHg). This is of particular interest as the correlation of IL-10 has been previously emphasized by Woiciechowsky et al., who analyzed the role of increased intracranial pressure (ICP) and sympathetic activation on systemic immune changes in animals [25]. They assessed plasma levels of the anti-inflammatory cytokine, interleukin IL-10 and showed that an increase in ICP was able to induce systemic release of IL-10. In this context Shiozaki et al. recently reported, that patients with an unfavorable outcome 6 month after TBI had elevated CSF cytokine levels 6 hours after TBI [12]. Maier et al. reported on a series of 29 patients, of whom two deceased [26]. Although they observed a not significant increase of IL-10 in CSF, he did not differentiate between surviving and deceased patients and did also not report, at which time point patients died. However, we found significantly elevated CSF IL-10 levels on admission of patients, who died within the first 24 hours after TBI.

Concerning serum IL-levels we also observed a significant increase of IL-10 in group II at 12 hours after

TBI. 24 hours after injury in the remaining two patients this level even elevated. In contrast patients in the survivor group presented a slight increase of IL-10 serum levels on admission. However, in comparison to control patients this was statistically not significant and already 12 hours after TBI IL-10 levels in survivors decreased again. This is in line with several other authors, also observing only slightly increased serum IL-10 following TBI [15, 17, 18]. Hensler et al., reported, that 4 hours after trauma IL-10 peaked in his collective [17]. In contrast Dziurdzik et al. observed no significant alterations of serum IL-10 [27]. He even did not observe differences between survivors and non-survivors. This seems to be in contrast to our data. But analyzing the study protocol the samples were taken on ICU admission. Comparing this to our protocol, the drawback is obvious. We drew samples immediately after admission to our trauma shock unit, i.e. 90 ± 45min after TBI and also we observed no alteration of serum IL-10 in survivors 12 hours after TBI.

Blood Brain Barrier (BBB) Function

Regarding the source of CSF IL-10 we additionally assessed the function of the Blood-Brain-Barrier (BBB) and found in the non-survivor group II a significant alteration until 24 hours after TBI. However, on admission non-survivors revealed peak-levels of CSF IL-10 but also lowest levels of serum IL-10. Patients in the survivor group had only mild dysfunction of the BBB during the entire observation period. These findings are in line with Csuka et al. They also analyzed thee BBB function using $Q_{albumin}$ and also found no correlation of BBB dysfunction and intrathecal inflammatory markers [15].

Although these results may favor the view of an intracerebral IL-10 synthesis it is not clear, whether resident cells of the CNS or activated immune competent cells invading the brain from the periphery, are the source of cytokines found in the CSF of patients after TBI. Supportive for an intracerebral synthesis of Il-10 is previous work showing that resident glial cells have the ability to produce Il-10 after challenge with LPS and pro-inflammatory cytokines TNF-α and IL-6 [28]. However, IL-10 measured in CSF may have a longer half-life compared to the protein present in serum since small peptides in peripheral circulation are rapidly sequestered by the liver [29]. The mechanisms mediating the passage of Il-10 through the even intact BBB by either active transport or diffusion processes need to be elucidated.

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