Raised parenchymal interleukin-6 levels correlate with improved outcome after traumatic brain injury

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Summary

Previous studies have suggested that an increased production of the pro-inflammatory cytokines interleukin-6 (IL-6) and interleukin-1β (IL-1β) can influence patient outcome following a severe head injury. However, these studies have relied upon measurements of cytokine levels in CSF or serum, rather than the brain parenchyma itself. Recently, a method of intracranial microdialysis has been developed which permits the efficient recovery of macromolecules from the parenchyma. We have used this technique to investigate whether there is a correlation between patient outcome and parenchymally derived cytokines. Fourteen patients who were admitted to the Wessex Neurological Centre with severe head injury were selected for the study. This group of patients consisted of seven males and seven females with an age range of 21–77 years. Patients were treated according to standard protocols including emergency craniotomy where necessary. Microdialysis probes were implanted into the frontal region contralateral to the site of the primary injury. Approximately 200 μl of dialysate was recovered every 8–12 h, and the concentrations of IL-6, IL-1β and nerve growth factor (NGF) were determined by commercial enzyme-linked immunosorbent assays. Patients were assessed initially using the Glasgow coma score, and survivors were assessed after 6 months using the Glasgow outcome scale. Significantly (P = 0.04) higher levels of IL-6 were found in patients who survived compared with those who died. Also, there was a significant correlation between peak IL-6 levels and Glasgow outcome scores (r² = 0.34, P = 0.03, n = 14). The levels of IL-1β and NGF were similar in both groups of patients. From these data, we suggest that IL-6 is an endogenous neuroprotective cytokine produced in response to severe head trauma.

Keywords: microdialysis; cytokine, neuroinflammation; traumatic brain injury (TBI); interleukin-6

Abbreviations: IL-1β = interleukin-1β; IL-6 = interleukin-6; NGF = nerve growth factor; TBI = traumatic brain injury

Introduction

Trauma is the leading cause of death in people under 45 years of age worldwide and, within this group, up to half of the fatalities are caused by head injury (Gennarelli, 1993; Jennett, 1998). The initial physical trauma to the brain parenchyma that occurs at the time of impact is followed by a series of dynamic processes that occur over a time span of hours or even days, which can influence neuronal and axonal survival (Mattson and Scheff, 1994; Tymianski and Tator, 1996).

The primary mechanical deformation of the brain following a severe head injury causes focal contusions and lacerations, and diffuse axonal injury. A subsequent series of secondary events will be initiated which perpetuates the neuronal and axonal injury induced by the primary injury. The importance of these dynamic secondary processes in traumatic brain injury (TBI) patients can be deduced from observations of the variability in patient outcome after apparently similar primary injuries (Teasedale, 1998). The secondary events initiated in TBI include excessive glutamate release, pathological elevation of the intracellular calcium concentration, production of free radicals and lipid peroxidation (Marshall, 2000). Further, there is a rapid, sustained elevation of pro-inflammatory cytokines and neurotrophins in the CNS, molecules that have been recognized as mediators of both neurodegenerative and neuroprotective mechanisms in a number of CNS pathologies (Arvin et al., 1995; Barone
An understanding of the roles of cytokines in the injured human brain has been hampered by an inability to measure cytokine concentrations within the brain parenchyma. As a consequence, our knowledge is restricted to measurements of cytokine levels in the CSF or serum of patients and experimental animals or in vitro studies. However, we reported recently (Winter et al., 2002) that it is possible to recover high molecular weight molecules from the parenchyma of the frontal lobe of the brain using a concentric microdialysis probe with a plasmapheresis membrane of molecular mass cut-off of 3000 kDa. Here we report a study of 14 patients with TBI in whom possible correlations were sought between patient outcome and changes in the concentrations of two candidate cytokines, interleukin-1β (IL-1β) and interleukin-6 (IL-6), and the neurotrophin, nerve growth factor (NGF), recovered from the parenchyma of the frontal lobe by microdialysis.

**Material and methods**

**Patient population**

The trial was granted ethical approval by the Southampton and SW Hampshire Local Regional Ethics Committee (LREC number 168/00) and studies were performed in accordance with the Declaration of Helsinki. No patient was entered into the study protocol without prior consent signed by an appropriate relative. Patients under the age of 16 years were excluded. The care or management of the patients was unaffected by involvement in the trial. The patient outcomes were assessed at 6 months after the head injury using the Glasgow outcome score where 5 = good recovery; 4 = moderate disability; 3 = severe disability; 2 = vegetative state; and 1 = death.

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Patients have been divided into two groups based upon their outcome. Patients in group A had a good recovery with Glasgow outcome scores (GOS) of 4 or 5, while those in group B all died in NICU (GOS = 1). GCS = Glasgow coma score; CT = classification of injury; ASDH = acute subdural haematoma; CDS = compound depressed skull fracture; Cont = contusions; DAI = diffuse axonal injury; EDH = acute extradural haematoma; ICH = intracerebral haematoma.

**Methods**

The method of intracerebral insertion and the details of the microdialysis apparatus have been described previously (Winter et al., 2002). Briefly, the microdialysis probe was constructed by Microbiotech, Stockholm, Sweden on our behalf using an Asahi-Plasmaseparator polyethylene polymer tubular membrane of internal diameter 330 µm and wall thickness 50 µm (Asahi Plasmaseparator, Diamed Medizitechnik, Cologne, Germany), with a molecular mass cut-off of 3000 kDa and an effective dialysis length of 12–15 mm. The microdialysis probe was inserted into the right frontal region, unless there was a contraindication such as a craniotomy flap compromising the stability of the intracerebral bolt, a compound depressed skull fracture or an underlying focal parenchymal injury (contusion or intracerebral haematoma). In these cases, the left frontal area was used. In all cases, the probe was intentionally positioned away from the site of focal pathology such that the cytokine levels in the dialysates would be indicative of the neuroinflammatory response secondary to the diffuse-type damage. Following a scalp stab incision, the intracerebral bolt, from a Microsensor Skull Bolt Kit (Codman Ltd, Bracknell, Berkshire, UK), was screwed into the skull. The dura was incised and the
microdialysis catheter introducer, followed by the microdialysis probe, was passed into the brain parenchyma to an approximate depth of 2 cm from the cortical surface. Normal saline solution (0.9% NaCl, Maco Pharma, London, UK) was used as the perfusate, and a MAB 20 peristaltic pump (Microbiotech) was set at a flow rate of 1 μl/min. Dialysates were collected into vials on ice. The first vial, which represented the saline in the outflow system, was collected for 30 min and then discarded. The second vial collected dialysate for ~3.5 h before being stored at −80°C. The pump was then switched off for 5–8 h before the next collection was made. The microdialysis probe was removed from the patient on day 6 (to reduce the risk of infection) or earlier if the patient was extubated. Blood samples were taken at the same time as the dialysis samples and the sera frozen at −80°C. All equipment was sterilized with ethylene oxide. A venous blood sample was also taken from a subset of the patients for cytokine analysis. All dialysates and serum samples were analysed for IL-6, IL-1β and NGF using commercial Ultrasensitive ELISA (enzyme-linked immunosorbent assay) kits (Biosource International, Camarillo, CA and Promega Corporation, Madison, WI). The minimal detectable levels of IL-6, IL-1β and NGF in the assays were 8, 3 and 16 pg/ml, respectively.

**Statistical analyses**

Because the data could not be assumed to be normally distributed, groups were compared using the non-parametric Mann–Whitney U test. Possible correlations were investigated using a linear regression model.

**Results**

**Patient details**

The clinical data from the 14 patients were analysed in conjunction with the individual cytokine and neurotrophin profiles. As shown in Table 1, the patients were divided into two groups based on their outcome, group A containing the nine survivors and group B the five patients who died on the NICU. The prevalence of acute subdural haematomas was higher in the non-survivors (four out of five patients) compared with the survivors (three out of nine patients), although a craniotomy was performed in approximately half the patients in each group. There was no evidence of infection in any of the patients, either at the site of implantation or systemically.

**Recovery of IL-6, IL-1β and NGF from the brain in vivo**

Figure 1 shows the temporal pattern of the recovery of IL-6 from brain extracellular fluid of all 14 patients. The survivors had significantly (P = 0.04) higher peak levels of IL-6 than the non-survivors (Fig. 2). There was a significant correlation between peak IL-6 levels and Glasgow outcome scores ($r^2 = 0.34$, $P = 0.03$, $n = 14$), while the trend towards a correlation between peak IL-6 levels and Glasgow coma scores failed to reach statistical significance ($r^2 = 0.23$,
The peak concentrations of IL-6, IL-1β and NGF recovered by microdialysis from nine surviving (closed symbols) and five non-surviving (open symbols) patients with TBI. Horizontal bars represent median values. IL-6 levels were significantly (P = 0.04) higher in survivors compared with non-survivors, but there was no correlation between outcome and IL-1β or NGF. The minimal detectable levels of IL-6, IL-1β and NGF were 8, 3 and 16 pg/ml, respectively.

P = 0.08, n = 14). Examination of the cumulative data suggested that peak levels of IL-6 occurred later in group A (54.6 ± 5.6 h) than group B (34.0 ± 10.9 h), although the difference was not statistically significant (P = 0.08).

The peak levels of IL-1β and NGF are also shown in Fig. 2. There were no obvious temporal trends in the levels of IL-1β and NGF. There was no significant difference between peak levels of IL-1β in the two groups and no associations between IL-1β levels and clinical measures. There was a tendency for peak NGF levels to be higher in non-survivors, but the difference was not statistically significant. However, ratios of peak levels of NGF : IL-6 were significantly (P = 0.01) lower in the survivors compared with the non-survivors. The median values (with range) were 1.4 (0.5–3.1) for the nine survivors and 19.2 (2.1–139) for the five non-survivors. Also, there were significant negative correlations between NGF : IL-6 ratios and both Glasgow outcome scores (r² = 0.28, P = 0.05, n = 14) and Glasgow coma scores (r² = 0.29, P = 0.05, n = 14).

In parallel to measuring parenchymal cytokine levels, we also determined the concentrations of IL-6 and IL-1β in the serum of a subset of patients. Temporal analyses revealed that there were no differences in the time at which peak serum cytokine levels were reached between the groups. The mean peak serum concentrations of IL-6 were 78 ± 20 pg/ml (n = 7) in survivors and 93 ± 9 pg/ml (n = 4) in those patients who died. The difference was not statistically significant. Levels of IL-1β were generally close to or below the limit of reliable detection (3 pg/ml). Estimates of mean peak serum concentrations were 5 ± 3 pg/ml (n = 7) for the survivors and 0.5 ± 0.3 pg/ml (n = 4) for non-survivors.

Discussion

Using microdialysis, we recovered IL-6, IL-1β and NGF from the frontal lobe parenchyma of 14 TBI patients, nine of whom survived and five of whom did not. Levels of IL-6 were significantly higher in the survivors. There were no significant differences between the groups in either dialysate concentrations of IL-1β and NGF or serum concentrations of IL-6 and IL-1β.

The levels of IL-6 measured in out-flowing dialysis fluid from the patients with TBI ranged between 60 and 1230 pg/ml, NGF between 180 and 2964 pg/ml, with lower levels of IL-1β, ranging between 0 and 140 pg/ml. We do not have comparable values of parenchymal cytokine levels in non-head-injured controls as we considered it to be unethical to perform microdialysis in such individuals. The levels of cytokines in the dialysis fluid are likely to be significant underestimates of the true extracellular concentrations in TBI for two reasons. First, the recovery efficiencies of our dialysis probes in vitro are ~45% for IL-6, 27% for IL-1β and 22% for NGF (Winter et al., 2002). Secondly, although it has not been studied in the brain, in other tissues prolonged dialysis leads to a partial depletion of dialysable molecules in the immediate vicinity of the probes (Clough et al., 2002). Consequently, although it is scientifically legitimate for us to cite only the cytokine levels present in the dialysate, local extracellular concentrations are likely to be at least double if not considerably more. Thus, our data compare favourably with reports of relatively high levels of cytokines in the plasma and CSF of head-injured patients. Increased IL-6 levels have been reported in the plasma (McClain et al., 1991; Kossmann et al., 1996; Singhal et al., 2002). It is likely that these elevations are the result of enhanced central production of the cytokine since IL-6 levels are significantly higher in the CSF (Kossmann et al., 1995, 1996; Maier et al., 2001; Muller et al., 2001) and levels in the jugular vein are significantly higher than those in the carotid artery (McKeating, 1997). Both IL-1β and NGF are also elevated in the serum and CSF of TBI patients (Patterson et al., 1993; Kossmann et al., 1996; Morganti-Kossmann et al., 1997; Singhal et al., 2002), with significantly higher levels occurring in the CSF. Levels of IL-1β, IL-6 and NGF are barely detectable in the plasma and CSF of healthy individuals and are normally virtually absent from the adult CNS.

Microdialysis probes were placed in the frontal lobes of the brain some distance from the focal damage. As molecules do not diffuse freely throughout the brain (Westerink and De Vries, 2001), it is unlikely that the cytokines recovered by microdialysis were generated by the area of focal damage. Also, for the reasons described in our previous publication (Winter et al., 2002), it is highly unlikely that cytokine generation stimulated by trauma due to the implantation of the microdialysis probes contributes significantly to the levels measured in the dialysate. Thus, it is likely that increased cytokine levels in the frontal lobe provide a biochemical marker of an endogenous response to secondary diffuse-type damage occurring throughout the brain. This conclusion would support the view of Marshall (2000) that the secondary processes in TBI are of great importance in determining clinical outcome.
A strength of microdialysis is its ability to monitor fluctuations in cytokine concentrations in individual patients at different times and between patients in the same study. Of particular note in this study was that concentrations of IL-6 recovered from survivors were significantly higher than those from non-survivors and also there was a significant correlation between peak IL-6 levels and Glasgow outcome scores in patients at 6 months. The lower IL-6 levels in non-survivors are unlikely to be a consequence of a global reduction in protein synthesis since there were no parallel falls in the concentrations of NGF. Also, the raised IL-6 levels in survivors are unlikely to arise from a systemic production as there was no difference between serum levels of IL-6 between the two groups. What this study cannot determine, however, is whether raised IL-6 levels are contributory to a good outcome or merely a consequence of the reparative processes in the recovering brain.

In humans, studies that have attempted to correlate IL-6 production with patient outcome following TBI have produced inconsistent results. Singhal et al. (2002) reported that elevated IL-6 levels were linked to good outcome, while Kalabiliikis et al. (1999) found no correlation between raised IL-6 and outcome. In contrast, other studies have suggested that elevated IL-6 in the serum or CSF is associated with poor outcome (Arand et al., 2001), possibly by suppressing a neuroprotective hypothermia (Aibiki et al., 1999). In animal models, studies of the role of IL-6 in brain injury have produced similarly inconsistent results. Intracerebroventricular injection of IL-6 is neuroprotective in a rat model of ischaemic stroke (Loddick et al., 1998), implying direct neuroprotective effects. Also, IL-6 knockout mice have reduced antioxidant capacity (Penkowa et al., 1999, 2000) and a slower rate of recovery following cortical injury (Swartz et al., 2001). Conversely, it has been reported that neuronal survival following optic nerve crush injury is significantly improved in IL-6-deficient mice (Fisher et al., 2001). In vitro, N-methyl-d-aspartate (NMDA) toxicity and oxygen–glucose deprivation in organotypic hippocampal slice cultures are commonly used to model excitotoxicity and cerebral ischaemia, the two primary mechanisms of neuronal death following traumatic brain injury (Vornov et al., 1994; Newell et al., 1995; Strasser and Fischer, 1995; Pringle et al., 1997; Wilde et al., 2000). In these preparations, which preserve many of the features of the in vivo hippocampus including an integrated network of neuronal and glial cells, and reproduce the regional vulnerability to a number of neurodegenerative stimuli (Vornov et al., 1991; Pringle et al., 1997), we have shown that IL-6, in similar concentrations to those recovered by microdialysis, is neuroprotective (Pringle et al., 2002).

In conclusion, we report the recovery by microdialysis of IL-6, IL-1β and NGF directly from the frontal lobe parenchyma of severely head-injured patients. Levels of IL-6 in microdialysis fluid, but not in serum, were significantly higher in patients who had a good recovery at 6 months compared with those who did not survive. From our data, a possible causal relationship between raised IL-6 levels and clinical recovery may be proposed. If such a relationship is proven by subsequent studies, it will open the door for the development of strategies for therapeutic intervention based on IL-6 in TBI patients.

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