Inhibition of Neurogenic Inflammation Attenuates the Inflammatory Response Following Traumatic Brain Injury in Rats

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Summary

A profound inflammatory response is initiated following traumatic brain injury (TBI). It has been proposed that serum IL-6 levels may serve as a marker for the severity of the injury. Using the rat impact-acceleration model of TBI, the study examined whether drugs which are able to inhibit neurogenic inflammation (capsaicin, NK1 antagonist), might influence the post-traumatic inflammatory response. In non-treated animals, TBI resulted in a significant increase in serum IL-6 levels. However, in animals pre-treated with capsaicin prior to injury, or treated with an NK1 antagonist following injury, this rise in IL-6 levels was not observed. We conclude that the inhibition of neurogenic inflammation may attenuate the inflammatory reaction associated with TBI, and help improve outcome.

Introduction

Traumatic brain injury (TBI) results in a profound inflammatory response within the central nervous system, which is characterised by the increased production of a number of pro-inflammatory mediators, including tumour necrosis factor alpha, interleukin-1beta and interleukin-6 (IL-6) (1). These mediators are considered to have both detrimental and beneficial effects in terms of neural outcome following TBI. On one hand they contribute to neuronal cell death and dysfunction, whilst on the other they establish the foundation for reparative processes. Whilst the tissue

levels of all these mediators increase following TBI, these changes are not directly reflected by changes in serum levels (2). Of these mediators, IL-6 is the only one where raised serum levels are readily detected following TBI. It has been proposed that increased blood levels of IL-6 following trauma may correlate with severity of injury and outcome (3, 4).

We have recently demonstrated that neurogenic inflammation plays a significant role in the early response to TBI (5). The aim of the present study was to examine whether agents that can modulate neurogenic inflammation have any affect on the subsequent cellular inflammatory response, and in particular, post-trauma serum levels of IL-6. Two different approaches were used to modulate neurogenic inflammation. Animals were either subject to pre-treatment with capsaicin, which results in a depletion of the peptide neurotransmitters, such as substance P, that are normally released from nociceptive nerves, or post-injury treatment with an NK1 tachykinin receptor antagonist. Two levels of injury were also examined. Animals were subject to either severe closed head injury, using the impact-acceleration model, or severe closed head injury followed by 30 minutes of hypoxia and hypotension.

Materials and Methods

Adult male Sprague-Dawley rats (350-450gms) were used in this study. One group (n=20) were pre-treated with capsaicin over a 3-day period (25, 50 and 50mg/kg), 14 days prior to injury or sham-injury, whilst the second group (n=20) received an NK1 antagonist (N-acetyl tryptophan; 1:mol/kg) 30 min after injury or sham injury. Severe TBI was induced using the impact-acceleration model of diffuse axonal injury as previously described in detail (6). Animals were anesthetized with isoflurane, and a stainless steel disc fixed to the exposed skull. The animal was then placed on a 10cm deep foam bed and injury induced by dropping a 450 gm brass weight a distance of two meters onto the disc. Animals were killed by decapitation at 6 hours after injury, and serum samples were collected.

For those animals also subject to hypoxia and hypotension following injury (n=10), they were mechanically ventilated using a Harvard Rodent Ventilator, whilst a femoral catheter enabled continuous mean arterial blood pressure monitoring (MABP). Severe TBI was induced as described above, immediately followed by the secondary insults which were maintained for 30min. Hypoxia was achieved by reducing inspired oxygen content to produce a PAO_2 between 30-40mmHg, whilst hypotension (MABP approx. 40mmHg) was induced by increasing the isoflurane and NO_2 concentrations. Animals were randomly assigned to receive injury with or without post-injury treatment with an NK1 antagonist (n=5/group). Animals were killed at 6 hours post-injury, and serum samples collected.

Serum levels of IL-6 were determined using a sandwich enzyme-linked immunoassay (ELISA) using a monoclonal antibody specific for rat IL-6 (R&D Systems, Minneapolis Mn). Statistical analysis was performed using the "Prism" statistics program (GraphPad Software, SanDiego Ca). Significance was determined using a one-way analysis of variance (ANOVA), with post-test analysis using Newman-Keuls multiple comparison test. A *p* value of less than 0.05 was considered significant.

Results

In studies examining the effects of severe TBI (Fig. 1a), the sham-injured animals exhibited low serum levels of IL-6 (103.7 ± 14.7pg/ml; n=10). For those animals subject to injury without drug treatment, there was a significant rise in serum IL-6 levels following severe TBI (289.3 ± 75.9pg/ml; $p < 0.01$). However, in animals that received pre-treatment with capsaicin, there was no significant difference in IL-6 levels as compared to the sham-injured animals (92.7 ± 26.7pg/ml). Similarly, those animals that received an NK1 antagonist 30mins after injury, showed no significant rise in serum IL-6 levels (127.6 ± 11.7pg/ml).

In studies examining the effect of secondary insults due to hypoxia and hypotension following severe TBI (Fig. 1b), the control animals again showed an apparent elevation in serum IL-6 levels following injury (585.4 ± 403.1pg/ml; n=5). However, those animals that received an NK1 an-

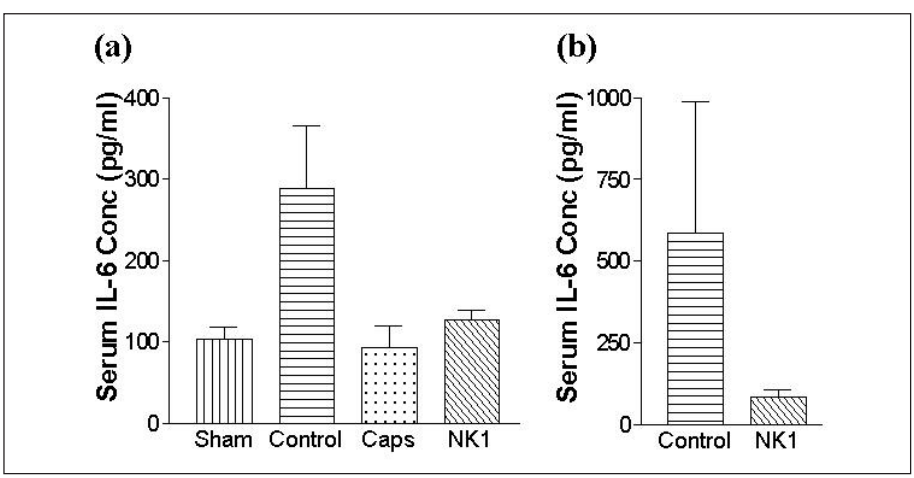

Figure 1. The effect of severe TBI (a), and severe TBI followed by 30 mins hypoxia and hypotension (b) on serum IL-6 concentrations. Animals were subject to either sham injury (Sham), TBI with no treatment (Control), TBI following pre-injury treatment with capsaicin (Caps), or TBI plus post-injury treatment with an NK1 antagonist (NK1).

tagonist following injury did not show an elevation in IL-6 levels despite the double insult (82.4 ± 24.4pg/ml). Behavioural observations also indicated that there were marked differences between the control and treated groups. Following hypoxia, the time to spontaneous breathing was less in the group treated with the NK1 antagonist (8.2 ± 0.9 mins versus 47 ± 33 mins; n=5; $p < 0.05$). Similarly, the time to animals demonstrating purposeful behaviour (feeding, grooming, drinking) was significantly shorter in the treated group (1.4 ± 0.2 hrs versus 4.2 ± 0.5 hrs; $p < 0.05$).

Conclusion

Severe TBI will result in a significant rise in serum IL-6 levels. However, our results indicate that drugs, which are able to modulate neurogenic inflammation, may prevent this rise in IL-6 levels. Pre-treatment with capsaicin, which inhibits the role of the nociceptive nerves in neurogenic inflammation, prevents post-trauma increases in IL-6 levels. However, capsaicin cannot be used as an interventional treatment, since its acute effects are pro-inflammatory. On the other hand, post-injury treatment with an NK1 tachykinin receptor antagonist, which prevents the action of substance P, also prevents the post-TBI rise in serum IL-6 levels. These results suggest that neurogenic inflammation may influence the subsequent cellular inflammatory reaction associated with TBI, and that inhibition of neurogenic inflammation may attenuate the inflammatory reaction within the CNS. Given that serum IL-6 levels may be indicative of injury severity, and likely outcome, these results suggest that the inhibition of neurogenic inflammation may be of clinical benefit in TBI.

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