The Effect of Human Corticotrophin Releasing Factor on the Formation of Post-Traumatic Cerebral Edema

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Summary

Controlled cortical impact is a well validated model of cortical contusion which is known to produce cerebral edema. Corticotrophin Releasing Factor (CRF) is a hypothalamic neuropeptide, which is known to inhibit transendothelial leakage of plasma derived fluid and tissue edema in response to injury. The aim of this study was to determine cerebral edema after controlled cortical impact and then compare the effect of high and low doses of CRF. We evaluated the effect of CRF in rats divided into groups of sham, trauma alone, and trauma treated with CRF at 50 micrograms/kg and 100 micrograms/kg. Animals were sacrificed at 24 hours and water content was determined. We found that CRF was effective in reducing cerebral edema associated with cortical contusion and propose that the action of CRF obviated barrier leakage.

Keywords: Cerebral edema; corticotrophin releasing factor; traumatic brain injury.

Introduction

Formation of cerebral edema following traumatic brain injury is a primary cause of morbidity and mortality. Edema formation may lead to a critical rise in intracranial pressure with concomitant fall in cerebral perfusion pressure, which in turn propagates further cerebral injury. The high mortality in those patients with sustained elevations of ICP and the poor prognosis of those patients who survive is well documented [1]. For this reason it is important clinically to limit edema formation, which can be seen both with diffuse and focal injuries.

Corticotrophin Releasing Factor (CRF) is a 41 amino acid hypothalamic neuropeptide, with a principle role in the hypothalamic-pituitary-adrenal axis. Recently this oligopeptide has been observed to limit edema formation in various models of tissue injury characterised by transendothelial leakage of plasma constituents [2–6], including surgical incision of muscle, cold injury to the cerebral cortex [2], thermal or neurogenic inflammation of the rat paw skin [3], and epinephrine or formaldehyde induced pulmonary edema [4].

Controlled cortical impact is a well validated model of cortical contusion, which is known to produce cerebral edema. The aim of this study was therefore to assess the ability of human CRF (hCRF) to reduce edema formation following craniocerebral trauma utilising the controlled cortical impact model of cortical contusion.

Methods

A dose escalation study of the effects of hCRF was employed, using doses of 50μg/kg and 100μg/kg hCRF. Human CRF was provided by Neurobiological Technologies Ltd, Richmond, Ca. A pH 4.0, 20mM acetate buffer with 5% mannitol was used as a vehicle for dilution. Drug dilutions were prepared on the morning of use, and stored refrigerated. All diluted drug was discarded after 24 hours. All animals used received human care in compliance with the Guide for the Care & Use of Laboratory Animals” (NIH Publication No. 86–23, 1985).

Adult male Sprague Dawley rats weighing 340–400g were randomised into one of 4 experimental groups. Group I, Sham trauma (n = 6); Group II, Trauma alone (n = 6); Group III, Trauma with 100μg/kg Corticotrophin Releasing Factor (CRF) administered post-injury (n = 7) and Group IV, Trauma with 50μg/kg CRF post-injury (n = 6).

The method of controlled cortical impact has been described in detail elsewhere [7]. Animals were ventilated with a mixture of N₂O (66%), O₂ (33%) and halothane (1.5%). The rats’ heads were secured in a stereotactic frame and a 10mm craniotomy prepared to the right of midline overlying the parietal cortex. Cortical contusion was obtained using a constrained stroke pneumatic impactor which delivered a controlled cortical impact. The impactor was set to deliver a blow at 6m/s impact velocity to a depth of 3mm. Immediately after injury the craniotomy site was sealed with the bone flap, gelfoam and rapid drying cyanoacrylic
Drug was administered in four divided doses (25µg/kg and 12.5µg/kg each dose, respectively). The first dose was given 30 minutes post-injury, the second at 2 hours post-injury, and the third and fourth doses at two hour intervals after this. The total administered dose of hCRF was 50µg/kg and 100µg/kg. Drug was injected subcutaneously into the nuchal region of the animal. Hydration of the animals was maintained using 10ml of intraperitoneal 0.9% saline.

Edema formation was determined using the method of wet and dry weights. Animals were sacrificed at 24 hours post-injury. Assessment was made of three 3mm slices centred under the site of injury and divided by hemisphere. The wet weight of these pieces was determined and the tissue incubated to a constant weight in an oven at 95°C, whereupon the dry weight was measured. The mean time for this process was 5 days. From the wet and dry weights of the tissue the cerebral water content was calculated.

**Results**

The overall mortality rate for animals exposed to cortical contusion was 21.9%, of which only 3.1% (n = 1 animal) died later than the time of the first drug dose. Drug administration could not therefore be observed to significantly affect mortality.

In the right (traumatised) hemisphere, trauma induced increases of 0.97, 1.25 and 0.87% in slices 3, 4 and 5 respectively compared with sham (p < 0.005) (Fig. 1a). This effect was attenuated in both CRF treated groups, with a maximal effect seen using 50µg/kg hCRF, where the water increase over sham was 0.1, 0.25 and 0.18% respectively (p < 0.01 cf. trauma). The effect in the 100µg/kg group was much less marked and not significant. In the left (non-traumatised) hemisphere the effect although present, was less obvious (Fig. 1b). This was in part due to the much smaller increases in cerebral water in this uninjured hemisphere (0-0.4%). Overall it is clear that the 50µg/kg dose is most effective at reducing edema.

Combining the water contents of these slices for the injured hemisphere, the overall water content in the sham group was 77.59 ± 0.14%. Trauma resulted in a rise in water content to 78.20 ± 0.48% (p < 0.05 cf. sham), and administration of 50µg/kg of hCRF resulted in a reduced water content of 77.62 ± 0.27% (p < 0.01 cf. trauma). 100µg/kg hCRF however did not attenuate the edema formation resulting in a water content of 78.08 ± 0.38% (Fig. 2).

ICP in the untreated injured animals rose from a baseline of 7.9 ± 2.9mmHg (mean ± sd), to a peak value of 41.4 ± 15.7mmHg in 3.6 ± 1.8mmHg minutes after trauma and then declined to a new steady state baseline of 23 ± 6.9mmHg, in 24.0 ± 17.4 minutes. ICP in the groups treated with hCRF did not show any significant difference to the untreated group.