Blood-brain Barrier Ultrastructural Changes in Impact Acceleration Head Trauma

M. Ghabriel[*], C. Zhu[*], A. Imran[*], P. Blumbergs[†] and P. Reilly[‡]

Departments of Anatomical Sciences[*], Pathology[†] and Surgery[‡], University of Adelaide, Adelaide, Australia 5005.

Summary

In a rat impact acceleration model of diffuse traumatic brain injury, oedema appears to be the principal factor in brain swelling. Here we aimed to investigate short-term blood-brain barrier alterations, 1-12h postinjury. A continuum of ultrastructural changes compatible with increased permeability was seen in brain endothelial cells at 1h, 3h and 6h, correlating with previous functional studies that suggested the development of early vasogenic oedema. Astrocytic swelling appeared at 6h and became pronounced at 12h, when most endothelial cells appeared normal, suggesting a shift towards cellular (cytotoxic) oedema.

Introduction

In closed head trauma, primary neurological damage is often worsened by secondary brain swelling and increased intracranial pressure. A rat model of impact acceleration head trauma was recently described (2, 5). In this model, oedema appears to be the major contributor to brain swelling (3) and is thought to be a combination of vasogenic and cellular oedema (4). Functional studies using radioactive tracers (6) and MRI studies (1) of this model suggest the occurrence of immediate vasogenic oedema but later more widespread cellular oedema. However, ultrastructural changes in the blood-brain barrier (BBB), particularly in the early phases postinjury, have not been fully elucidated. The aim of the current study was to determine short-term ultrastructural changes in the BBB in this model, 1h-12h after trauma.

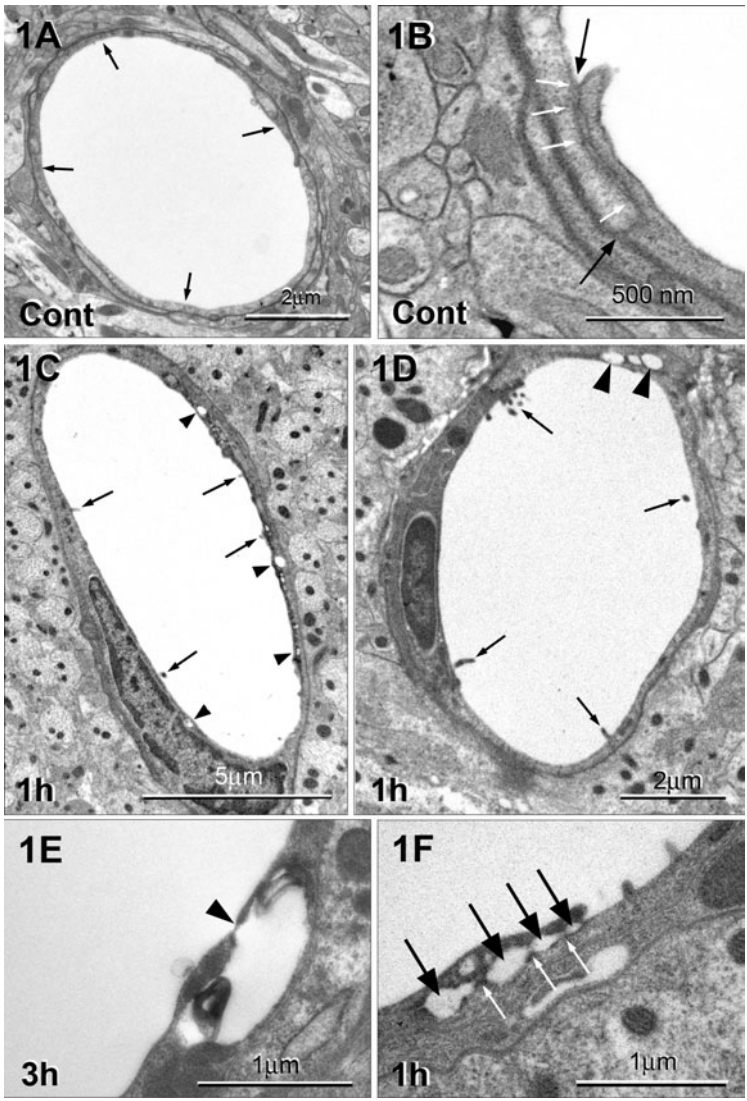

Figure 1. *Electron micrographs of cerebral capillaries from control (Cont, 1A and B) and experimental (1C-F) animals, 1 and 3h postinjury.* ***1A.*** *Cerebral capillary showing thin endothelial cells (arrows) with absence of luminal microvilli and large vesicles. There is no obvious extracellular space.* ***1B.*** *Part of a capillary wall showing long oblique inter-endothelial tight junction (between black arrows). The tight junction shows points of fusion (white arrows) of the apposed membranes.* ***1C - E.*** *Endothelial cell luminal membranes show microvilli-like projections (arrows) and the cytoplasm shows small vesicles (small arrowheads) and large vacuoles (large arrowheads).* ***1F*** *shows a compromised tight junction with focal cleft widening (black arrows) and separation of the points of membrane fusion (white arrows).*

Materials and Methods

Sprague-Dawley male rats (400-500g) were anaesthetised by isoflurane inhalation via endotracheal tube. A metallic disc was fixed to the exposed intact skull using quick adhesive. The animal was place on a soft foam bed and impact acceleration head trauma was induced by 450g weight falling freely onto the metal disc from height of 2m (2, 5). Animals were allowed to recover until electively killed by cardiac perfusion with 4% paraformaldehyde-2% glutaraldehyde fixative. Two animals each were used at 1h, 3h, 6h and 12h postinjury, and two normal animals as control. Brain tissue was examined by electron microscopy.

Results

Brain vessels in control animals had thin endothelial cells (ECs) with few cytoplasmic vesicles and closed tight junctions. The extracellular space was indistinct (Fig. 1A and B). In experimental animals at 1h and 3h, ECs showed numerous cytoplasmic vesicles, luminal membrane projections (Fig. 1C and D) and crater-like invaginations. Large vacuoles occupied the full thickness of ECs (Fig. 1E), often adjacent to tight junctions. Tight junctions between ECs were open (Fig. 1F). At 6h, many ECs still displayed the above changes (Fig. 2A), in addition to the appearance of perivascular spaces and swelling of perivascular astrocytic endfeet (Fig. 2B). Astrocytic swelling became maximal at 12h, but most ECs now appeared normal (Fig. 3).

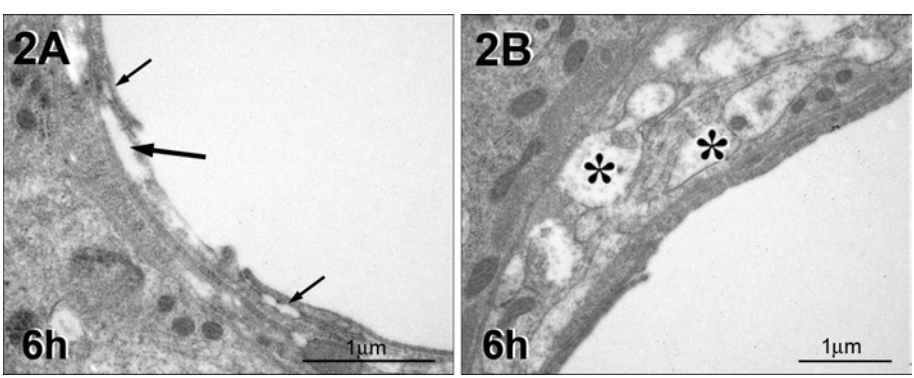

Figure 2. *Electron micrographs of cerebral capillaries from experimental rats killed 6h postinjury.* ***2A*** *shows three endothelial cells and two widened tight junctions (small arrows). The middle endothelial cell shows a large cavity (large arrow).* ***2B*** *shows early swelling (asterisks) in perivascular astrocytes.*

Conclusions

The present study of an impact acceleration head trauma model dem-

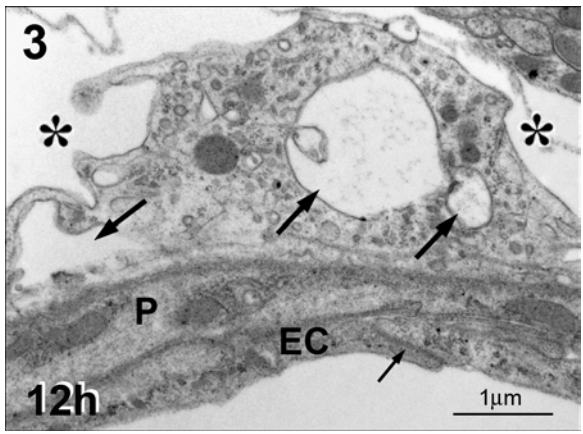

***Figure 3**. Electron micrograph of cerebral microvessel, 12h postinjury showing endothelial cells (EC) with closed tight junction (small arrow) and a pericyte (P). A perivascular astrocyte shows large cavities (large arrows). The extracellular space is widened (asterisks).*

onstrated early ultrastructural changes in brain endothelial cells, with opening of tight junctions. These changes support functional studies that suggest the development of early vasogenic oedema. Astrocytic (cytotoxic) oedema as indicated by swelling of astrocytes, appeared at 6h and became pronounced at 12h. By that time most ECs appeared normal.

References

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