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Induction of apolipoprotein E after traumatic brain injury in forensic autopsy cases

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Abstract We investigated the dynamics of the induction of apolipoprotein E (apoE) in the human brain after death caused by traumatic brain injury (TBI). A striking difference in apoE immunoreactivity in the traumatised cortical hemisphere compared with the contralateral non-traumatised hemisphere was observed. ApoE was detected within the neurons of the traumatised cortical hemisphere in cases surviving only about 2 h, as well as in those surviving for extended periods. In contrast, no apoE staining within the neurons was seen in the contralateral cortical hemisphere. ApoE staining within astrocytes was faint in both traumatised and contralateral hemispheres of cases surviving only 2 h. However, staining was intense in the traumatised hemispheres in short as well as long surviving cases, even those surviving more than 1 month. ApoE immunoreactivity was also observed in areas adjacent to capillaries and surrounding the neuropil of the injured hemisphere. These observations corroborated the idea of a prolonged induction of apoE within the neurons and also in the extracellular matrix after TBI. Furthermore, the possibility is suggested that the alteration of apoE distribution may contribute to a cerebroprotective mechanism immediately after TBI.

Keywords Apolipoprotein E · Traumatic brain injury Immunohistochemistry · Astrocyte · Neuron

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Introduction

There are three isoforms of apolipoprotein E (apoE) in humans, encoded by three different alleles, ε2, ε3 and ε4 (E2, E3 and E4) and it is thought that the presence of the epsilon (ε) 4 allele may predispose to neurological disease. For example, it is known that the frequency of apoE-E4 is significantly higher in late-onset familial and sporadic Alzheimer's patients than in controls [24]. In addition, clinical investigations show that isoforms of apoE are prognostic factors after TBI [1, 3, 4, 5, 15, 17, 18, 25, 26, 29]. Thus, apoE is closely associated with certain CNS disorders, but the precise mechanisms for this have not yet been elucidated.

ApoE is produced mainly in astrocytes, but not in neurons [2] and is essential for lipid transport and metabolism. It is proposed that ApoE regulates synaptic remodelling and cytoskeletal interactions in the developing or injured brain by mediating transport of cholesterol and lipoprotein [20, 21, 28]. Animal studies have shown that the cellular distribution of apoE is altered in brain disorders. Selective uptake of apoE by astrocytes and dying neurons after transient cerebral ischemia in the rat has been reported [12, 13] and apoE immunoreactivity was identified in vulnerable neurons following transient brain ischemia [6, 16]. Moreover, localisation of apoE to the cytoplasm of human neurons was demonstrated in senile dementia or Alzheimer's disease [7, 8]; thus, the possibility that neurons produce apoE under some pathological conditions cannot be excluded. Localisation of apoE in neurons may therefore have significant pathophysiological implications.

Consequently, using forensic autopsy cases, we investigated alterations in the cellular distribution of apoE immunoreactivity in the human brain after TBI, in an attempt to establish whether there was a significant role of apoE in the response to TBI.

Table 1 Clinical and autopsy data from cases following TBI (*Fr* fracture, *SDH* subdural hemorrhage or hematoma, *SAH* subarachnoidal hemorrhage, *CC* cerebral contusion, *ICH* intracerebral hemorrhage, *H* herniation, *l* left, *r* right)

Case	Age (years)	Sex	Survival time	Post-mortem period (h)	Trauma	Lesions	Additional clinical and autoptical data
1	13	F	Instantaneous death	14	Fall	Frontoparietal r	Fr, IHC
2	18	M	Instantaneous death	8	Fall	Frontotemporal l	Fr, SAH
3	54	M	1 h	13	Fall	Frontoparietal l	SAH, H
4	32	M	1.5 h	20	Traffic accident	Frontotemporal r	Fr, CC
5	43	M	2 h	16	Traffic accident	Occipital l	Fr, SAH, CC, ICH
6	61	M	4 h	11	Fall	Frontobasal l	Fr, SDH
7	56	M	5 h	20	Assault	Parietooccipital r	Fr, SAH, CC, ICH
8	66	M	7 h	4	Assault	Frontoparietal r	SAH, CC
9	63	M	8 h	6	Fall	Frontal l	Fr, CC
10	32	M	8 h	11	Assault	Frontobasal l	SAH, CC
11	57	M	9 h	5	Assault	Frontotemporal r	Fr, SDH, SAH
12	41	F	10 h	18	Assault	Frontobasal l	SDH, CC
13	61	M	12 h	11	Fall	Frontotemporal l	Fr, SAH
14	45	M	18 h	20	Assault	Frontal r	Fr, SDH, SAH, CC
15	52	M	24 h	24	Fall and assault	Frontal l	Fr, SDH, CC
16	58	M	2 days	2	Fall and assault	Parietotemporal r	SDH, SAH, H
17	49	F	2 days	16	Assault	Frontotemporal l	SDH, SAH, CC, ICH
18	20	F	3 days	60	Fall	Frontotemporal l	Fr, SDH, SAH, H
19	22	M	3 days	11	Traffic accident	Parietotemporal l	Fr, SDH, SAH, CC
20	48	M	4 days	12	Assault	Temporal r	SDH, SAH, CC, ICH
21	42	M	5 days	4	Fall and assault	Frontotemporal r	SDH, CC, H
22	65	F	5 days	13	Assault	Frontotemporal l	SDH, CC, H
23	59	F	6 days	6	Fall and assault	Frontobasal r	SDH, H
24	73	M	7 days	12	Fall	Temporobasal r	SDH, SAH, H
25	55	F	7 days	17	Assault	Temporal r	SDH, CC, H
26	76	F	8 days	20	Assault	Frontotemporal l	SDH, SAH, ICH
27	35	M	9 days	16	Assault	Parietal l	SAH, ICH
28	19	M	10 days	3	Assault	Frontotemporal l	SDH, CC, H
29	70	F	14 days	18	Assault	Frontotemporal r	SDH, SAH, CC
30	31	M	23 days	24	Fall	Frontobasal l	Fr, SDH, H
31	75	F	1 month	15	Assault	Frontotemporal l	Fr, SDH, H
32	60	M	3 months	24	Assault	Parietotemporal r	SDH
33	72	M	5 months	6	Assault	Frontotemporal r	SDH

Table 2 Clinical and autopsy data from control cases

Case no.	Age (years)	Sex	Post-mortem period (h)	Cause of death
C1	51	M	16	Exsanguination due to a stab wound to the liver and the inferior vena cava
C2	69	M	20	Exsanguination due to a stab wound to the branches of the mesenteric arteries
C3	58	M	12	Exsanguination due to rupture of the esophageal varices
C4	43	M	20	Exsanguination due to a stab wound to the intercostal artery and the left lung
C5	28	M	11	Exsanguination due to a stab wound to the pul monary artery, the heart and the left lung
C6	60	F	20	Ligature strangulation
C7	18	F	17	Ligature strangulation
C8	39	F	6	Ligature strangulation
C9	20	M	16	Manual strangulation
C10	63	M	24	Atypical hanging
C11	58	M	7	Atypical hanging
C12	47	F	9	Typical hanging

Materials and methods

For this study 33 cases with traumatic brain injury (23 males and 10 females) were studied using material from forensic autopsies. The age range was 13–76 years (mean age 49.2 years) and the post-mortem interval ranged from 2 h to approximately 60 h. Survival times varied from instantaneous death to death 5 months after injury. All the cases had local lesions of the brain, including cranial bone fracture, subdural hemorrhage (hematoma), subarachnoidal hemorrhage, cortical contusion, intracerebral hemorrhage and herniation (Table 1). As a control group 12 cases (8 males and 4 females) without brain injury were also examined where death was due to exsanguination in 5 cases and in the remaining 7 to mechanical asphyxia (Table 2).

Tissue blocks from the traumatised cerebral cortex and also from the contralateral non-traumatised cerebral cortex were fixed in formalin and embedded in paraffin. Thin serial sections of 8 μm were then cut. Hematoxylin and eosin (HE) staining as well as Klüver-Barrera (KB) staining was routinely performed on sections from all tissue blocks. For comparison, serial sections were used for immunohistochemical staining as well as conventional staining.

The following antibodies were used for the immunohistochemical study: polyclonal goat antiserum to human apoE (diluted 1:500; Calbiochem, La Jolla, Calif.), anti-GFAP monoclonal antibody as a marker of astrocytes (diluted 1:200; Dako, Copenhagen, Denmark) and anti-CD68/PG-M1 monoclonal antibody as a marker of microglia (diluted 1:200; Dako, Copenhagen, Denmark). Immunoreactive apoE, GFAP and CD68/PG-M1 were localised using the avidin-biotin-peroxidase (ABC) method. Biotinylated anti-mouse and goat IgG antibodies and Vectastain "Elite" ABC kits were obtained from Vector Laboratories (Burlingame, Calif.). Microwave antigen retrieval was used for apoE and PG-M1. For each case, omission of the primary antibody was used as a negative control and no positive staining was seen in any of these control sections. In addition, in order to confirm the specificity of anti-apoE antibodies, monoclonal anti-apoE antibody (anti-apoE monoclonal antibody; Transduction Laboratories, Lexington, USA, 1:5,000) was employed immunohistochemically for selected cases (cases 3, 5, 15, 20, C1, C7 and C11).

The sections were assessed microscopically and the pathological changes documented under conditions ensuring that the pathologist was unaware of each case history. This study was carried out according to the ethical guidelines of Nagasaki University, School of Medicine.

Results

Monoclonal antibody against apoE generally showed a weaker reaction than the polyclonal antibody. Of the immunohistochemical markers used, monoclonal antibody against apoE and polyclonal antiserum reacted very much in parallel (data not shown).

ApoE induction could not be detected in control cases without traumatic brain injury, or within 1.5 h after TBI (cases 1–4, Fig. 1). However, weak apoE immunoreactivity was observed in neurons and the surrounding injured cerebral cortex of the traumatised hemisphere in case 5, where survival after injury was only 2 h. Slight apoE immunoreactivity was also detected in astrocytes. In contrast, the cerebral cortex contralateral to the injury showed no positive apoE staining in neurons and slight apoE staining only in a small number of astrocytes in this case (Fig. 2).

In a case surviving for 4 h (case 6), intensive immunoreactivity of apoE was found within neurons of the traumatised cerebral cortex directly beneath the injury and also within astrocytes in the deep part of the cortex adjacent to the location of the hemorrhage. In addition, apoE was found to be accumulated in the deep part of the cortex contiguous to the capillaries in the traumatised hemisphere. No apoE immunoreactivity was detected in neurons or in the deep cortex contiguous to capillaries in the contralateral hemisphere, although very faint apoE staining was observed in astrocytes deep in the cortex, much fainter than in the injured side. In the cases with 5 h–10 days survival (cases 7–28, Fig. 3), a similar distribution of apoE immunoreactivity was observed within neurons and astrocytes over a comparable area. However, the area positive for apoE was wider and also more numerous positive cells were detected. The apoE immunoreactivity was heavily concentrated in the area surrounding necrotic tissue at the site of injury and in the deep cortex adjacent to such damaged sites. Likewise, in these cases, patchy distribution of apoE immunoreactivity was observed in the neuropil surrounding the capillaries, tending to increase with longer survival times (Fig. 4). In contralateral hemispheres, no apoE immunoreactivity was detected in neurons, although very faint apoE staining was observed in a few astrocytes in the deep part of the cortex. Furthermore,

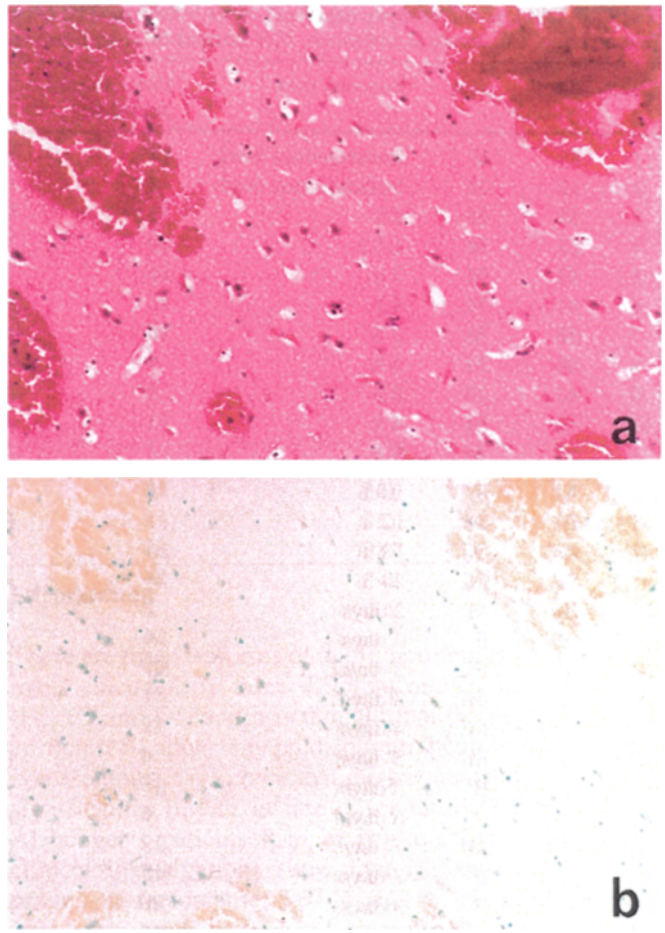

Fig. 1 A case surviving for 1 h after head injury (case 3 in Table 1, ×50), **a** HE staining, **b** immunostaining for apoE

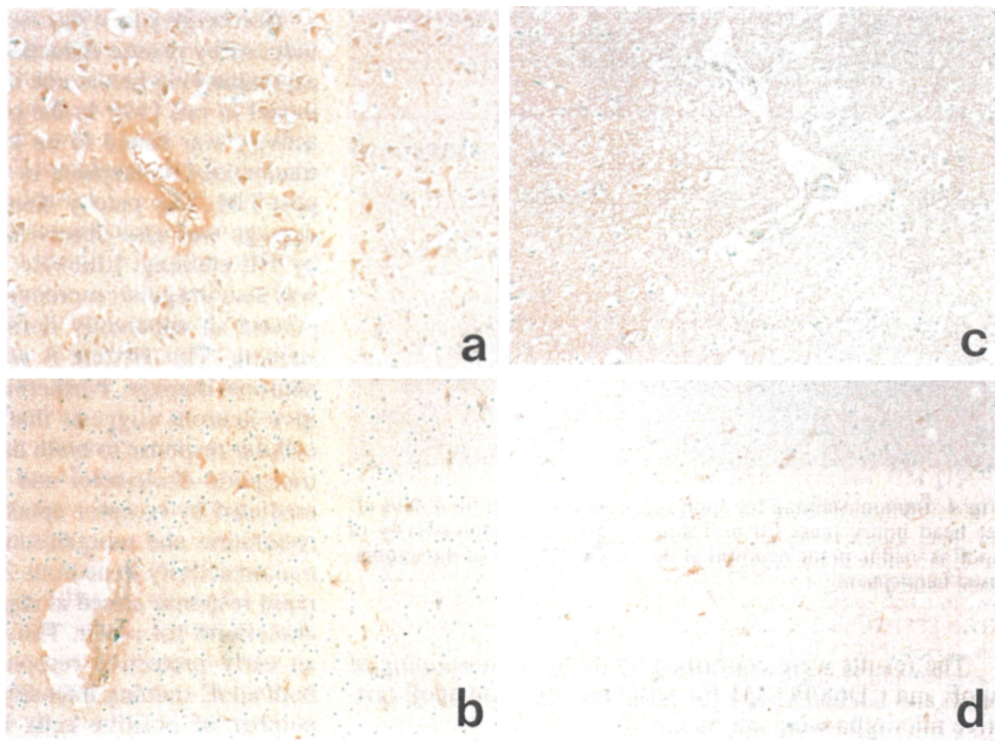

Fig. 2 a–d Immunostaining for apoE in a case surviving for 2 h after head injury (case 5 in Table 1, ×50). **a** Cerebral cortex of the traumatised hemisphere, **b** deep part of the cerebral cortex of the traumatised hemisphere, **c** cerebral cortex contralateral to injury and **d** in the deep part of the cerebral cortex contralateral to injury

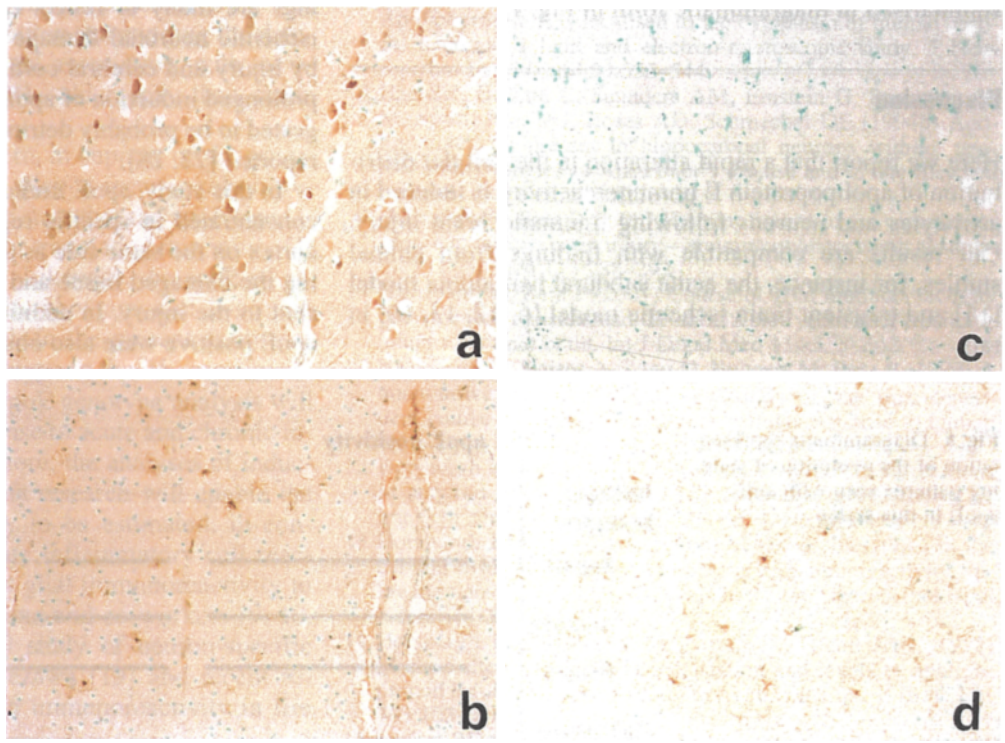

Fig. 3a–d Immunostaining for apoE in a case surviving for 24 h after head injury (case 15 in Table 1, ×50). **a** Cerebral cortex of the traumatised hemisphere, **b** in the deep part of the cerebral cortex of the traumatised hemisphere, **c** cerebral cortex contralateral to injury and **d** in the deep part of the cerebral cortex contralateral to injury

no apoE immunoreactivity was seen in the interstitial tissue contiguous to capillaries or in the neuropil surrounding capillaries.

In cases surviving more than 10 days after injury (cases 29–33), although the cellular distribution of apoE positivity in the injured hemisphere was similar to that of cases surviving only 4 h, the level of reactivity was markedly decreased both in terms of staining intensity and positive cell count. However, intensive apoE immunoreactivity was observed in the interstitial tissue adjacent to capillaries and in the surrounding neuropil. In contrast, apoE immunoreactivity was scarcely observable in astrocytes of the hemisphere contralateral to injury. Moreover, apoE was also negative in astrocytes in the contralateral hemisphere in cases surviving more than 23 days after TBI (cases 30–33).

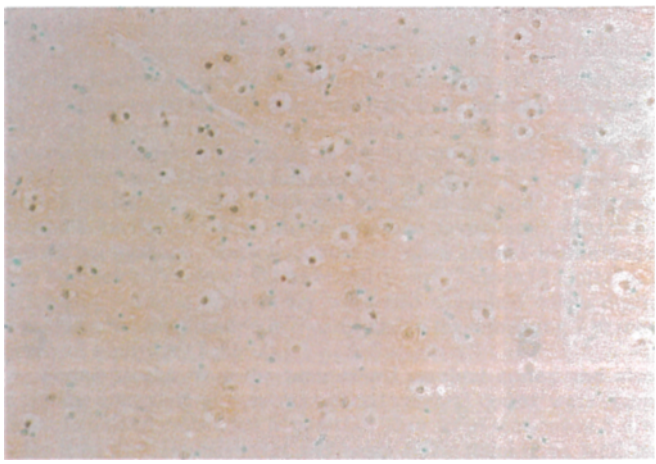

Fig. 4 Immunostaining for apoE in a case surviving for 4 days after head injury (case 20 in Table 1, ×50). Immunoreactivity of apoE is visible in the neuropil of the cerebral cortex of the traumatised hemisphere

The results were confirmed by the immunostaining of apoE and CD68/PG-M1 for serial sections and apoE positive microglia were not identified.

These findings, with survival times for all cases, are summarised in diagrammatic form in Fig. 5.

Discussion

Here we report that a rapid alteration in the cellular distribution of apolipoprotein E immunoreactivity is induced in astrocytes and neurons following traumatic brain injury. Our results are compatible with findings from animal studies, for instance, the acute subdural hematoma model [14] and transient brain ischemic model [6, 12, 13, 16] in rats.

Horsbergh stated that ischemic neuronal cell damage is induced by drastic reduction of local cerebral blood flow accompanying hematoma in the acute cerebral hematoma model in rats [14]. In the present study, apoE immunoreactivity was found to be localised in the neurons in the traumatised hemisphere in cases surviving more than 2 h post-TBI. The patchy distribution of ischemic neuronal damage was also observed in the corresponding regions by HE staining. Likewise, apoE localisation in neurons was also irregular; moreover, apoE immunoreactivity was present in apparently normal neurons according to HE staining. This finding is assumed to represent reversible neuronal damage. Furthermore, the presence of apoE positive neurons suggests that apoE may play a role in the cellular response to brain damage. It is reported that apoE transports cholesterol and lipid into cellular cytoplasm, mediated by receptor uptake, for the purpose of synaptic restoration and reorganisation [20]. Therefore, apoE immunoreactivity in neurons 2 h after injury may represent a rapid response aimed at supplying damaged neurons with cholesterol for repair. Thus, this phenomenon constitutes an early protective response to brain damage. Further, both apoE staining intensity within neurons as well as the number of positive cells in the traumatised region increased with time up to 10 days after injury. These findings are likely to reflect chronological augmentation of ischemic neuronal damage at the site of necrosis caused by injury and cerebral cortical hemorrhagic lesions. Such prolonged induction of apoE within neurons has been suggested to be probably detrimental to neurons as previously reported [23, 28].

In this study, apoE immunoreactivity in astrocytes was concentrated in specific regions as follows: the cerebral cortex on the same side as the injury, the region surrounding the damaged tissue and the deep cerebral cortex adjacent to the injury. In addition, the astrocytes which were apoE positive were also observed in the corresponding re-

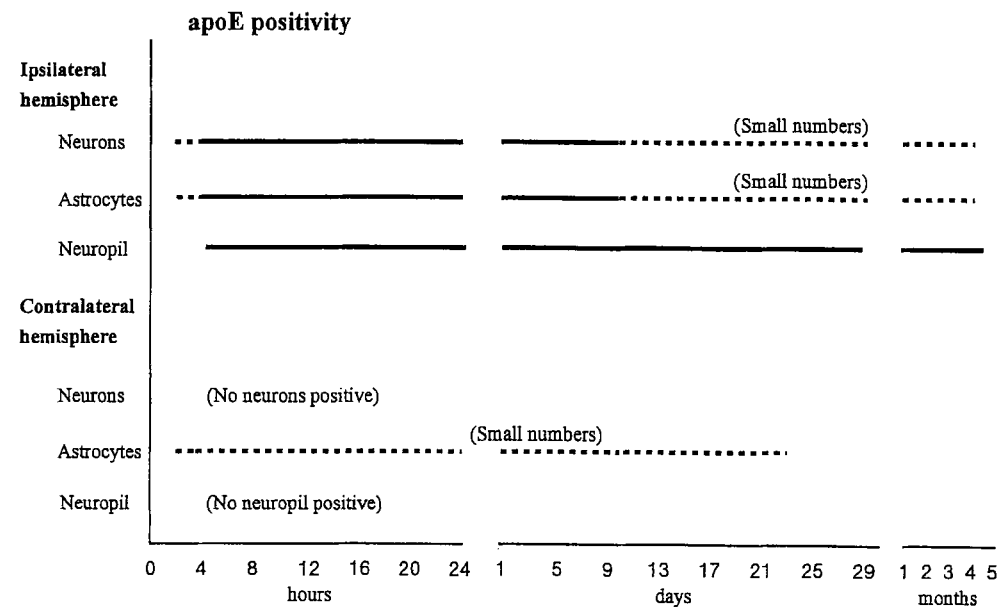

Fig. 5 Diagrammatic representation of the evolution of staining patterns seen with anti-apoE in this series

gion on the other side. However, apoE immunoreactivity in the contralateral deep cerebral cortex was weaker than that on the same side as the injury. The apoE immunoreactivity in astrocytes is likely to be related to stress and damage to neurons, because there was a close relationship between the localisation of apoE immunoreactivity in neurons and the density of apoE induction in astrocytes at the injured site. Nonetheless, in the contralateral hemisphere, apoE immunoreactivity was not identified within neurons but was observed only in astrocytes although the pathophysiological significance of this finding remains obscure.

In the present investigation, apoE immunoreactivity was documented in the interstitial tissue adjacent to capillaries and in the neuropil surrounding capillaries in the injured hemisphere. This finding indicates that apoE immunoreactivity was present extracellularly. This was observed from 4 h after injury onwards and a striking accumulation and widespread distribution of apoE immunoreactivity was found in longer survival cases, even up to 5 months after injury. This implies that the observed association between such extracellular apoE deposition and the microcirculation results from disruption of the blood-brain-barrier by injury, allowing apoE egress. Further, it has been reported that maximum cerebral blood flow is observed surrounding cerebral contusion [30]. In the present study, localisation of apoE immunoreactivity was concentrated in the microcirculation surrounding the damaged tissue which suggests an association with cerebrovascular dilatation after TBI, which may then cause secondary brain damage.

There have been some reports in support of the possibility that apoE isoforms may play a specific role in recovery from CNS insult. These state that the apoE4 genotype predicts poor outcome after traumatic brain injury [1, 3, 4, 5, 15, 17, 18, 25, 26, 29]. Likewise, the apoE ε4 allele is associated with deposition of amyloid β-protein following head injury [17, 29]. In the present study, we did not determine the apoE subtype in the 45 cases investigated. In a future study, the apoE genotype analysis will possibly enable the CNS response to acute and chronic injury to be elucidated. Furthermore, the analyses of induction of apoE localisation in this research will enable the precise time from brain injury to be estimated, in conjunction with results from acute inflammatory cell reaction, the vascular response and glial immunoreactivity to human brain injury by Hausmann and co-workers [9, 10, 11], the immunohistochemical study of neuron-specific enolase by Ogata and Tsuganezawa [19] and recent research of intranuclear ubiquitin immunoreactivity in fire fatalities by Quan et al. [22].

In summary, our data provide evidence for alterations of the intra- and extracellular localisation of apoE immunoreactivity as a pathophysiological mechanism after TBI. These findings may reflect a role for apoE in neuronal restoration and reorganisation. We consider that apoE is an essential biological mediator of the cellular response of the CNS to TBI. Moreover, a precise analysis of apoE induction will be valuable for elucidation of neuronal pathophysiological conditions after brain injury.

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