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Interleukin (IL)-8 immunoreactivity of injured axons and surrounding oligodendrocytes in traumatic head injury

Takahito Hayashi *, Kazutoshi Ago, Takuma Nakamae, Eri Higo, Mamoru Ogata *

Department of Legal Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan

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ABSTRACT

Interleukin (IL)-8 has been suggested to be a positive regulator of myelination in the central nervous system, in addition to its principal role as a chemokine for neutrophils. Immunostaining for beta-amyloid precursor protein (AβPP) is an effective tool for detecting traumatic axonal injury, although AβPP immunoreactivity can also indicate axonal injury due to hypoxic causes. In this study, we examined IL-8 and AβPP immunoreactivity in sections of corpus callosum obtained from deceased patients with blunt head injury and from equivalent control tissue. AβPP immunoreactivity was detected in injured axons, such as axonal bulbs and varicose axons, in 24 of 44 head injury cases. These AβPP immunoreactive cases had survived for more than 3 h. The AβPP immunostaining pattern can be classified into two types: traumatic (Pattern 1) and non-traumatic (Pattern 2) axonal injuries, which we described previously [Hayashi et al. Int. J. Legal Med. 129 (2015) 1085–1090]. Three of 44 control cases also showed AβPP immunoreactive injured axons as Pattern 2. In contrast, IL-8 immunoreactivity was detected in 7 AβPP immunoreactive and in 2 non-AβPP immunoreactive head injury cases, but was not detected in any of the 44 control cases, including the 3 AβPP immunoreactive control cases. The IL-8 immunoreactive cases had survived from 3 to 24 days, whereas those cases who survived less than 3 days ($n = 29$) and who survived 90 days ($n = 1$) were not IL-8 immunoreactive. Moreover, IL-8 was detected as Pattern 1 axons only. In addition, double immunofluorescence analysis showed that IL-8 is expressed by oligodendrocytes surrounding injured axons. In conclusion, our results suggest that immunohistochemical detection of IL-8 may be useful as a complementary diagnostic marker of traumatic axonal injury.

1. Introduction

Diffuse traumatic axonal injury is an important cause of traumatic coma in the absence of an intracranial mass lesion [1]. In forensic practice, the recognition of traumatic axonal injury is particularly important, since it signifies a rotational injury of considerable severity as may be observed in a road traffic accident, a fall from a height, or an accelerated blow by a punch. However, it is difficult to diagnose traumatic axonal injury in postmortem examination because macroscopic changes may be minimal or even trivial, and most changes can only be recognized microscopically [2]. Under the electron microscope, ultrastructural changes, such as focally enlarged axons with axolemmal infolding or disordered neurofilaments, can be observed within fields of axons exhibiting neither apparent macroscopic nor light microscopic abnormalities at 6 h survival after head injury [3,4].

Immunostaining for a protein transported along axons, beta-amyloid precursor protein (AβPP), is the most effective tool for detecting traumatic axonal injury in forensic practice [1,2,5–11]. AβPP immunoreactivity can detect injured axons as axonal bulbs, varicose axons (sinusoidally swollen axons), and wavy axons as early as 2–3 h after head injury [1,2,10–12]. Moreover, some axonal bulbs can be detected at shorter survival times after head injury depending on the case [13,14]. However, AβPP immunoreactivity also detects axonal injuries due to hypoxic insults [1,10–17]. Several previous studies, including ours [1,17–20], have described the different patterns of AβPP immunoreactivity between traumatic and non-traumatic (hypoxic) axonal injuries.

Interleukin (IL)-8, a glutamic acid-leucine-arginine (ELR) motif-positive chemokine (chemotactic cytokine), is a major chemotactic factor for neutrophils in acute inflammation [21,22]. Results of recent studies suggest that IL-8 also functions as a promoter of the myelination of injured axons by mediating the recruitment of oligodendrocyte progenitor cells [23,24]. Incomplete disruptive

* Corresponding authors. Tel.: +81 99 275 5313; fax: +81 99 275 5315.
E-mail addresses: takahito@m2.kufm.kagoshima-u.ac.jp (T. Hayashi), ogatam@m.kufm.kagoshima-u.ac.jp (M. Ogata).

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axonal injury, which usually occurs as diffuse axonal injury, causes a sequence of changes that result in secondary axotomy (complete axonal disconnection) [5]. The initial axon cytoskeletal changes, which result from calcium (Ca^{2+})-mediated ultrastructural collapse, may accompany the demyelination of axons, and, thus, the myelination of injured axons may be required for regeneration. Recent evidence also suggests that myelin debris (degradation products of myelin) from injured axons can trigger inflammation with the up-regulation of a variety of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-1β, and IL-6 [25]. In the present study, we compared IL-8 and AβPP immunoreactivities in the corpus callosum of deceased patients with blunt head injury and investigated whether the immunohistochemical detection of IL-8 is useful in the forensic diagnosis of traumatic axonal injury.

2. Materials and methods

2.1. Antibodies

The following monoclonal antibody (mAb) and polyclonal antibodies (pAbs) were used for immunohistochemical and immunofluorescence analyses in the present study: mouse anti-AβPP mAb (EMD Millipore, Darmstadt, Germany), mouse anti-IL-8 mAb (Santa Cruz Biotechnology, Inc., Dallas, TX, USA), rabbit anti-myelin basic protein (MBP) pAbs (Thermo Fisher Scientific, Waltham, MA, USA), biotinylated goat anti-mouse IgG (Santa Cruz Biotechnology, Inc.), cyanine dye 3 (Cy3)-conjugated donkey anti-mouse IgG pAbs, and fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG pAbs (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA).

2.2. Autopsy samples

Forty-four cases with blunt head injury (age range, 2–93 years; mean age, 61.0 years; 34 males and 10 females; range of survival time after injury, short time (<10 min) to 90 days) and the same number of control cases with hypoxia/brain ischemia without head injury (age range, 2–92 years; mean age, 54.9 years; 25 males and 19 females) were collected from the Department of Legal Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University. The details of the head injury and control cases are summarized in Tables 1 and 2, respectively. This cohort of head injury cases and control cases is identical to that examined in our previous study [20]. From each deceased patient, the corpus

Table 1
Details of the cases with head injury.

Case no.	Age range and gender	Survival time	Cause of head injury	Brain lesions	[a]AβPP	Pattern	[a]IL-8
1	80s M	90 d	Falling	SDH	++	?	–
2	90s F	24 d	Assault	SDH	+	1	++
3	80s M	21 d	Traffic accident	SDH, CC, ICH	–		–
4	80s M	18 d	Assault	SDH, SAH, CC	–		–
5	80s M	14 d	Assault	SDH, SAH	++	1, 2	+
6	60s M	12 d	Falling	SDH, SAH	++	1	–
7	40s M	9 d	Assault	EDH, SDH, SAH, CC	++	1, 2	++
8	30s M	7 d	Falling	SDH, SAH, CC	+	1	+
9	60s M	7 d	Falling	SAH, CC	+	?	–
10	60s M	7 d	Falling	SDH, SAH	–		–
11	30s F	6 d	Assault	SDH, SAH	–		+
12	60s F	5 d	Assault	SDH, SAH, CC	++	1	++
13	60s M	4 d	Assault	SDH	++	1, 2	+
14	2 M	3 d	Assault	SDH, SAH	++	1, 2	+
15	60s M	3 d	Falling	SDH, CC	–		+
16	50s M	2.5 d	Falling	SDH	+	?	–
17	40s M	2.5 d	Assault	SAH	–		–
18	70s M	2.5 d	Assault	SDH, SAH, CC	++	1, 2	–
19	20s M	32 h	Assault	SAH, CC	–		–
20	40s M	1 d	Falling	SDH, CC	+	1	–
21	30s M	1 d	Assault	SDH, SAH, CC	++	1, 2	–
22	50s M	1 d	Assault	SDH, SAH, CC	++	1, 2	–
23	50s M	14 h	Falling	EDH, SDH, SAH	+	1	–
24	30s M	13 h	Falling	SDH, SAH	–		–
25	50s M	12 h	Falling	SDH	+	1	–
26	50s M	9 h	Assault	SAH, CC	+	1	–
27	60s M	8 h	Falling	SDH, SAH, CC	–		–
28	70s M	8 h	Falling	SDH, SAH, CC	+	1	–
29	60s M	7 h	Falling	SDH, SAH	++	1	–
30	60s M	7 h	Assault	SDH, SAH, CC	–		–
31	80s F	6 h	Traffic accident	EDH, SDH, SAH, CC	+	1	–
32	80s M	6 h	Traffic accident	SAH	+	1	–
33	70s M	4.5 h	Traffic accident	SDH, SAH, CC	–		–
34	50s M	4 h	Falling	SDH, SAH, CC	+	1	–
35	90s M	3 h	Falling	SDH, SAH, CC	+	1	–
36	80s F	2.5 h	Falling	SAH	–		–
37	50s M	2 h	Falling	SAH, CC	–		–
38	70s F	1 h	Falling	SDH, SAH, CC	–		–
39	80s F	1 h	Falling	SDH	–		–
40	60s F	0.5 h	Assault	SAH	–		–
41	70s F	Short	Assault	SAH, CC	–		–
42	20s M	Short	Assault	SAH	–		–
43	70s M	Short	Falling	SAH	–		–
44	40s F	Short	Assault	SDH	–		–

EDH, epidural hematoma; SDH, subdural hematoma; SAH, subarachnoid hemorrhage; CC, cerebral contusion; ICH, intracerebral hemorrhage.
[a] -, 0 axonal bulbs (AB); +, 1–5 AB; ++, >5 AB per 200× microscopic field.